ION CHANNEL GENE VARIANTS PREDISPOSING TO SEVERE HUMAN VENTRICULAR ARRHYTHMIAS

Annukka Marjamaa

Research Program in Molecular Medicine,
and Department of Medicine and Department of Cardiology,
University of Helsinki, Finland
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Supervised by

Docent Heikki Swan
Department of Cardiology
University of Helsinki
Helsinki, Finland

Professor Kimmo Kontula
Department of Medicine
University of Helsinki
Helsinki, Finland

Reviewed by

Professor Johanna Kuusisto
Department of Medicine
University of Kuopio
Kuopio, Finland

Professor Terho Lehtimäki
Department of Clinical Chemistry
University of Tampere
Tampere, Finland

Official opponent

Professor Heikki Huikuri
Department of Internal Medicine
University of Oulu
Oulu, Finland

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LIST OF ORIGINAL PUBLICATIONS

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I Lehtonen A, Fodstad H, Laitinen-Forsblom P, Toivonen L, Kontula K, Swan H. Further evidence of inherited long QT syndrome gene mutations in antiarrhythmic drug-associated *torsades de pointes*. HeartRhythm 2007; 4(5):603-7.

II Marjamaa A, Salomaa V, Newton-Cheh C, Porthan K, Reunanen A, Karanko H, Jula A, Lahermo P, Väänänen H, Toivonen L, Swan H, Viitasalo M, Nieminen MS, Peltonen L, Oikarinen L, Palotie A, Kontula K. High prevalence of four long QT syndrome founder mutations in the Finnish population. Annals of Medicine 2009; 41(3):234-40.

III Marjamaa A*, Newton-Cheh C*, Porthan K, Reunanen A, Lahermo P, Väänänen H, Jula A, Karanko H, Swan H, Toivonen L, Nieminen MS, Viitasalo M, Peltonen L, Oikarinen L, Palotie A, Kontula K, Salomaa V. Common candidate gene variants are associated with QT interval duration in the general population. Journal of Internal Medicine 2009; 265(4): 448-58.

IV Marjamaa A, Laitinen-Forsblom P, Wronska A, Toivonen L, Kontula K, Swan H. Ryanodine receptor (*RyR2*) mutations in sudden cardiac death: studies in extended pedigrees and phenotypic characterization *in vitro*. Submitted.

V Marjamaa A, Laitinen-Forsblom P, Lahtinen AM, Viitasalo M, Toivonen L, Kontula K, Swan H. Search for cardiac calcium cycling gene mutations in familial ventricular arrhythmias resembling catecholaminergic polymorphic ventricular tachycardia. BMC Medical Genetics 2009; 10:12.

* Equal contribution

ABBREVIATIONS

aLQTS	acquired long QT syndrome
AMPK γ_2	gamma 2 regulatory subunit of AMP-activated protein kinase
ANK2	ankyrin-B gene
ARVC/D	arrhythmogenic right ventricular cardiomyopathy/dysplasia
ATP2A2	cardiac Ca^{2+} ATPase (SERCA2a) slow twitch gene
βAR	β-adrenergic receptor
bpm	beats per minute
CACNA1C	voltage-gated L-type calcium channel Cav1.2 alpha 1_C subunit gene
CAPON	neuronal nitric oxide synthase regulator
CASQ2/CASQ2	calsequestrin gene/protein
$Ca_v1.2$ α_{1c}	voltage-gated L-type calcium channel Cav1.2 alpha 1_C subunit protein
CAV3	caveolin-3
CICR	Ca^{2+}-induced Ca^{2+} release
cLQTS	congenital long QT syndrome
CPR	cardiopulmonary resuscitation
CPVT	catecholaminergic polymorphic ventricular tachycardia
DAD	delayed afterdepolarization
dHPLC	denaturing high-performance liquid chromatography
DNA	deoxyribonucleic acid
EAD	early afterdepolarization
ECG	electrocardiogram
FKBP12.6	calstabin protein
FKBP1B	calstabin gene
G3PD1L	glycerol-3-phosphate-dehydrogenase 1-like protein
GJA5	connexin 40 gene
GPD1L	glycerol-3-phosphate dehydrogenase 1-like gene
HERG/HERG	human ether a-go-go-related gene/protein
ICD	implantable cardioverter defibrillator
$I_{Ca,L}$	L-type calcium current
I_{K1}	inward potassium current
I_{Kp}	potassium plateau current
I_{Kr}	rapidly activated delayed rectifier potassium current
I_{Ks}	slowly activated delayed rectifier potassium current
I_{Kur}	ultrarapid potassium current
I_{Na}	sodium current
$I_{Na/Ca}$	sodium-calcium exchanger current
I_{ti}	transient inward potassium current
I_{to}	transient outward potassium current
JLNS	Jervell-Lange-Nielsen syndrome
KCNA5	potassium voltage-gated channel, shaker-related subfamily, member 5 gene
KCND	potassium voltage-gated channel, shal-related subfamily gene
KCNE1	potassium voltage-gated channel, Isk-related family, member 1 gene
KCNE2	potassium voltage-gated channel, Isk-related family, member 2 gene
KCNH2	potassium voltage-gated channel, subfamily H (ether a-go-go -related), member 2 gene
KCNJ2	potassium inwardly-rectifying channel, subfamily J, member 2 gene
KCNJ12	potassium inwardly-rectifying channel, subfamily J, member 12 gene
KCNK	potassium channel, subfamily K gene
KCNQ1	potassium voltage-gated channel, KQT-like subfamily, member 1 gene
Kir2.1 α	inward rectifier K^+ channel Kir2.1, alpha subunit
$K_v7.1$ α	voltage-gated K^+ channel $K_v7.1$, alpha subunit

$K_v11.1$ α	voltage-gated K^+ channel $K_v11.1$, alpha subunit
LQTS 1-10	long QT syndrome subtypes 1-10
LVEDD	left ventricular end-diastolic volume
minK β	voltage-gated potassium channel subunit beta
MiRP1 β	potassium channel subunit beta MiRP1; MinK-related peptide 1, beta subunit
MLPA	multiplex ligation-dependent probe amplification
$Na_v1.5$ α	voltage-gated sodium channel type V, alpha subunit
NCX1	cardiac Na^+/Ca^{2+} exchanger
NOS1	neuronal nitric oxide synthase 1
NOS1AP	neuronal nitric oxide synthase adaptor gene
NS	nonsignificant
PCR	polymerase chain reaction
PIRA	primer-induced reaction assay
PKA	protein kinase A
PRKAG2	AMP-activated protein kinase gamma 2 subunit gene
QT	QT interval
QTc	QT interval corrected for heart rate (Bazett)
QT_{Nc}	QT interval corrected for heart rate (nomogram)
RWS	Romano-Ward syndrome
RyR/RyR 1 and 2	ryanodine receptor gene/protein type 1 or 2
SCD	sudden cardiac death
SCN4B	voltage-gated sodium channel, type IV, beta
SCN5A	sodium channel alpha subunit gene
SERCA2a	sarcoplasmic reticulum Ca^{2+} ATPase
SLC8A1	solute carrier family 8 (sodium/calcium exchanger), member 1
SNP	single-nucleotide polymorphism
SOICR	store overload-induced Ca^{2+} release
SR	sarcoplasmic reticulum
SUD	sudden unexplained death
TdP	*torsades de pointes*
TDR	transmural dispersion of repolarization
VPC	ventricular premature complex
VT	ventricular tachycardia

ABSTRACT

Inherited cardiac arrhythmia disorders are rare but clinically important, as they underlie sudden cardiac deaths among otherwise healthy young individuals. Congenital long QT syndrome (LQTS) with an estimated prevalence of 1:2000-1:10 000 manifests with prolonged QT interval on electrocardiogram and risk for ventricular arrhythmias and sudden death. Several ion channel genes and hundreds of mutations in these genes have been identified to underlie congenital LQTS. In Finland, four LQTS founder mutations of potassium channel genes account for up to 40-70% of genetic spectrum of LQTS. Acquired LQTS has similar clinical manifestations, but often arises from usage of QT-prolonging medication or electrolyte disturbances. A prolonged QT interval is associated with increased morbidity and mortality not only in clinical LQTS but also in patients with ischemic heart disease and in the general population.

The principal aim of this study was to estimate the actual prevalence of LQTS founder mutations in Finland and to calculate their effect on QT interval in the Finnish background population. Using a large population-based sample of over 6000 Finnish individuals from the Health 2000 Survey, we identified LQTS founder mutations *KCNQ1* G589D (n=8), *KCNQ1* IVS7-2A>G (n=1), *KCNH2* L552S (n=2), and *KCNH2* R176W (n=16) in 27 study participants. This resulted in a weighted prevalence estimate of 0.4% for LQTS in Finland. Using a linear regression model, the founder mutations resulted in a 22- to 50-ms prolongation of the age-, sex-, and heart rate-adjusted QT interval. Collectively, these data suggest that one of 250 individuals in Finland may be genetically predisposed to ventricular arrhythmias arising from the four LQTS founder mutations. In a separate study, LQTS founder mutations were identified in a subgroup of acquired LQTS, providing further evidence that congenital LQTS gene mutations may underlie acquired LQTS.

Disease-causing LQTS mutations, although enriched in the Finnish population, are rare and cannot account for the population burden of severe ventricular arrhythmias. Even subtle changes in QT interval duration caused by common gene variants may be important at the population level. The effect of common LQTS gene variants on QT interval was studied in the Health 2000 material using linear regression. A *KCNE1* D85N minor allele with a frequency of 1.4% was associated with a 10-ms prolongation in adjusted QT interval and could thus identify individuals at increased risk of ventricular arrhythmias at the population level. In addition, the previously reported associations of *KCNH2* K897T, *KCNH2* rs3807375, and *NOS1AP* rs2880058 with QT interval duration were confirmed in the present study.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is characterized by exercise-induced ventricular arrhythmias in a structurally normal heart and results from defects in the cardiac Ca^{2+}

signaling proteins, mainly ryanodine receptor type 2 (*RyR2*). In a patient population of typical CPVT, *RyR2* mutations were identifiable in 25% (4/16) of patients, implying that noncoding variants or other genes are involved in CPVT pathogenesis. A 1.1 kb *RyR2* exon 3 deletion was identified in two patients independently, suggesting that this region may provide a new target for *RyR2*-related molecular genetic studies. Since CPVT is associated with high mortality, a search for *RyR2* mutations in a cohort of sudden cardiac death patients was performed. Two novel *RyR2* mutations (R3570W and G2145R) showing a gain-of-function defect *in vitro* were identified in three victims of sudden cardiac death. The two carriers of R3570W were distant relatives and showed mildly enlarged and dilated hearts at autopsy. None of the surviving *RyR2* R3570W carriers featured the exercise-induced ventricular arrhythmias typical of CPVT, but mild structural abnormalities and resting ventricular arrhythmias were apparent in a few relatives.

In conclusion, the four LQTS founder mutations leading to considerable QT interval prolongation appear to be exceptionally prevalent in the Finnish population and may provide a rationale for population screening for arrhythmia susceptibility in the future. In addition, common LQTS variants, *KCNE1* D85N in particular, appear to modulate QT interval duration at the population level. Not all *RyR2* mutations lead to a typical, highly penetrant CPVT phenotype, underscoring the relevance of tailored risk stratification of a *RyR2* mutation carrier.

INTRODUCTION

Cardiac ion channelopathies are a set of inherited disorders characterized by electrical instability of the heart, typically in the absence of structural heart disease. The spectrum of the disease phenotypes is wide, the most malignant arrhythmia syndromes being those that cause ventricular tachycardia. Although rare, the inherited ventricular arrhythmia syndromes are of clinical importance as they may underlie morbidity and mortality at a young age.

The last two decades have revolutionized knowledge of inherited arrhythmia disorders. The first clinical descriptions of familial arrhythmia disorders emerged in the late 1950s, when Jervell et al. and Levine et al. described pedigrees with unusually long QT intervals on electrocardiogram, structurally normal hearts and increased risk of sudden death (Jervell et al. 1957, Levine et al. 1958). However, the underlying causes remained unknown for decades. The linkage study by Keating et al. in 1991 identified the first long QT syndrome disease locus (Keating et al. 1991), and subsequent studies using a candidate gene approach and/or linkage analysis have led to the identification of several genes and hundreds of disease-causing mutations in cardiac ion channel disorders (Priori et al. 2008). Similar approaches have been used to unravel the genetic background of other inherited arrhythmia disorders. Unquestionably, the completion of the human genome project at the beginning of this decade (Lander et al. 2001, Venter et al. 2001) has laid the cornerstone for these recent advances in molecular medicine. Characterization of familial gene defects in these pedigrees enables the genetic testing of yet asymptomatic relatives, which is of high priority if the disorder is malignant and treatment available. In addition, identification of unusual genotype-phenotype correlations, new disease genes, and subsequent *in vitro* and murine studies on disease mechanisms have brought new insights into the biological processes underlying disease pathology and provide targets for therapeutic innovations.

The increasing phenotypic complexity underlying inherited cardiac ion channelopathies has overwhelmed researchers in the field since the first description of variable penetrance in long QT syndrome (LQTS) (Vincent et al. 1992). Apparently, both genetic and environmental factors interact with the particular pathogenic mutation and contribute to disease phenotype. Identification of modifying factors could have direct implications for risk stratification of a mutation carrier. Ultimately, these relatively common genetic variants could also identify individuals at increased risk for arrhythmias at the population level. The development of high-throughput genotyping techniques has enabled studies on large sample sizes powered to detect genetic modifying factors of arrhythmia susceptibility. Studies have not necessarily focused directly on disease phenotype, but utilized intermediate phenotypes, such as QT interval duration in LQTS. The aim of this study was to identify both rare and common genetic variants predisposing to severe ventricular arrhythmias in clinical samples and at the population level.

REVIEW OF THE LITERATURE

1 ELECTRICAL ACTIVITY OF THE HEART

1.1 Cardiac action potential

The heart is an electromechanical pump that depends on action potential generation and contraction, followed by relaxation and a period of refractoriness until the next impulse is generated (Nerbonne et al. 2005). Myocardial electrical activity is generated in the pacemaker cell of the sinoatrial node and then mediated through the atria and atrioventricular node to conducting Purkinje fibers and ultimately to the ventricular myocardium (Nerbonne et al. 2005). Cardiac excitability results from a highly coordinated balance of both depolarizing and repolarizing ion currents (Marban 2002). Cardiac ion channels that selectively mediate the flow of ions across biological membranes have variable expression in specific regions of the heart, thus resulting in a distinct action potential morphology (Roden et al. 2002). In ventricular myocytes, the action potential exhibits a sharp depolarizing upstroke in phase 0 due to influx of Na^+ (Figure 1) (Berne 1998), which is followed by K^+ efflux, producing early repolarization in phase 1. The slowly decaying plateau in phase 2 results from Ca^{2+} influx from the extracellular space and is involved in the excitation-contraction coupling that eventually leads to ventricular contraction (Berne 1998).

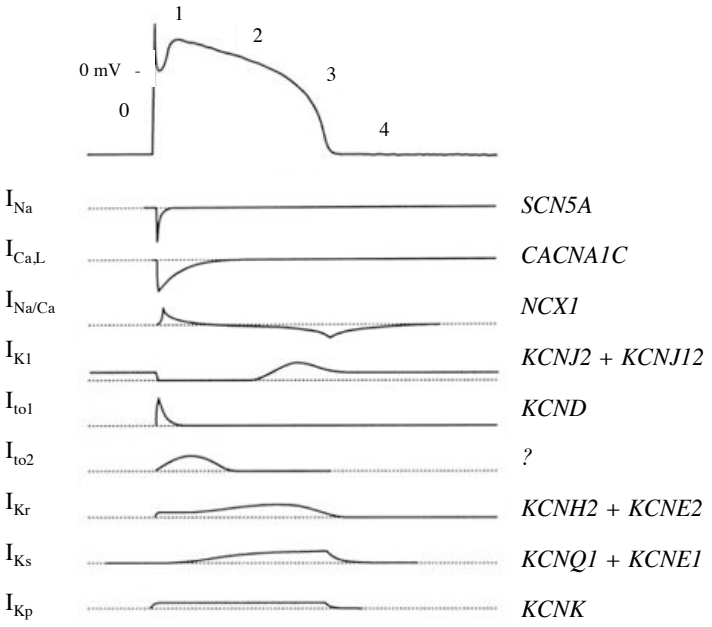

Figure 1. Cardiac action potential in ventricular myocytes comprising phases 0-4. Cardiac ion currents (left panel) and the genes (right panel) encoding the respective ion channels.

Cardiac repolarization is based on the intricate balance of predominantly outward K^+ currents (Berne 1998). The I_{K1} current is active at negative potentials and restores the baseline potential (Marban 2002). The transient outward current I_{to} is responsible for the initial notch of repolarization in phase 1, thus influencing the duration of the action potential (Marban 2002). The delayed rectifier I_K currents, composed of rapid (I_{Kr}) and slow (I_{Ks}) components, are the major determinants of final repolarization in phase 3 (Marban 2002). The restoration of ion concentrations is achieved by the action of Na^+K^+ ATPase and Na^+/Ca^{2+} exchanger, which pump Na^+ into the extracellular space and in exchange restore K^+ and Ca^{2+} ions in the cardiomyocyte (Berne 1998).

1.2 Cardiac excitation-contraction coupling

Calcium influx via voltage-gated L-type Ca^{2+} channels in phase 2 of the cardiac action potential initiates Ca^{2+} release from the sarcoplasmic reticulum, a phenomenon known as Ca^{2+}-induced Ca^{2+} release (CICR) (Bers 2002). The calcium release is transmitted via sarcoplasmic ryanodine receptor type 2 (RyR2) receptor complex, comprising several associated proteins such as triadin, junctin, and calsequestrin (Bers 2002). The elevated free intracellular Ca^{2+} concentration allows the binding of Ca^{2+} to troponin C, initiating the contraction of myofilaments (Bers 2002). The cardiac contraction is terminated by transmitting cytosolic free Ca^{2+} ions back to the sarcoplasmic reticulum (SR) via the SR Ca^{2+} ATPase (SERCA2a) pump (Bers 2002). In addition, the Na^+/Ca^{2+} exchanger and Ca^{2+} ATPase on the sarcolemma as well as a mitochondrial Ca^{2+} pump contribute to the elimination of free Ca^{2+}, and thus, the initiation of myocardial relaxation (Bers 2002).

1.3 Cardiac ion channels

Ion channels are pore-forming proteins that control a voltage gradient across the plasma membrane, resulting in either depolarization or hyperpolarization of the cell (Celesia 2001). Most ion channels are gated and classified according to the control mechanisms required for channel opening such as voltage, ligand binding, G-protein interaction, or mechanical gating (Felix 2000). Voltage-gated ion channels are critical for the appropriate electrophysiologic behavior of the heart. The cardiac ion channel subunits are each encoded by a single gene. The pore-forming α-subunit is often sufficient to generate an ion current, but the coordinated function of α-subunits, accessory β-subunits, and multiple modulating proteins is necessary for appropriate trafficking, phosphorylation, and posttranslational modifications of the channels (Roden et al. 2002). The α-subunit consists of six hydrophobic S1-S6 segments embedded in the plasma membrane (Felix 2000). The pore region resides between segments S5 and S6, while the highly conserved S4 contains several positive amino acid residues that function as the voltage sensor of the channel (Felix 2000). A tetramer of identical α-subunits is required to generate a functional voltage-gated K^+ channel with ion-permeant ion pores (Roden et al. 2002), while in Na^+ and Ca^{2+} channels the same channel structure consists of four repeats of S1-S6 transmembrane regions. In addition to the voltage-

gated cardiac ion channels, the inward rectifier K^+ channels are crucial for the electrical activity of the heart and present a more primitive channel structure, with two membrane-spanning segments and the intervening pore region (Roden et al. 2002).

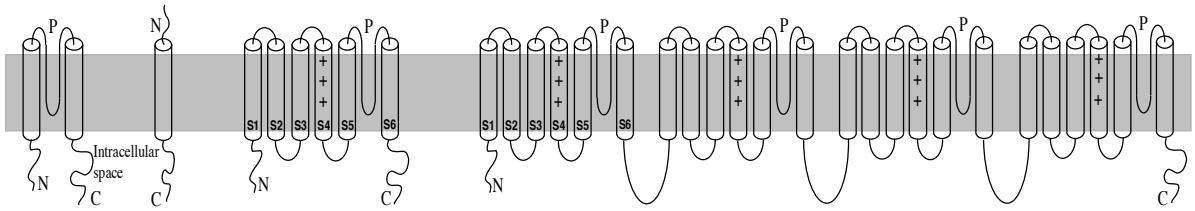

Figure 2. Schematic representation of the transmembrane topology of voltage-gated K^+, Na^+, and Ca^{2+} channels. The potassium channel is composed of four identical α-subunits, each comprising six transmembrane domains (S1-S6) and the interlinking regions. The S1-S6 segment of the potassium channel is homologous to the four Na^+ and Ca^{2+} channel domains. The more primitive K^+ channels consist of either one or two transmembrane segments. The amino (NH_2) and carboxy (COOH) termini are presented with N and C, respectively. P refers to pore-forming regions.

1.4 Mechanisms of arrhythmias

Re-entry and triggered activity are the primary mechanisms for disturbances in cardiac rhythm. In re-entry, the electric impulse re-excites a region that has previously been activated (Berne 1998). Triggered activity is caused by both early and delayed afterdepolarizations, EADs and DADs, respectively (Berne 1998). EADs occur at the end of the plateau (phase 2) or in phase 3, before the cell is fully repolarized, and are most likely to appear at low heart rates (Berne 1998). Prolonged action potential is hypothesized to allow the Ca^{2+} channels of the plateau phase to be reactivated and to trigger EADs (Berne 1998). The transmural dispersion of repolarization (TDR) of the myocardial wall and the development of EADs are the substrate for the *torsades de pointes* arrhythmia encountered in LQTS (Antzelevitch 2004). By contrast, DADs occur at relatively high heart rates and are associated with elevated intracellular Ca^{2+} concentrations (Berne 1998). The rise in intracellular Ca^{2+} causes a release of sarcoplasmic Ca^{2+}, which in turn activates Na^+ and K^+ passage into the cell (Berne 1998). The net effect of this transient inward current I_{ti} is DADs of the sarcolemma, leading to triggered activity (Schlotthauer et al. 2000).

1.5 Repolarization components on electrocardiogram

Disturbed cardiac excitability is detectable on surface electrocardiogram (ECG), which represents an average of the electrical gradients (Marban 2002). The QT interval, determined as the time from the onset of the QRS complex to the end of the T wave, shows the duration of ventricular depolarization and repolarization. The ventricular myocardium consists of several myocardial cell types with diverse

electrophysiological characteristics (Antzelevitch 2004). M cells present a smaller slowly activating delayed rectifier potassium current (I_{Ks}) and a larger late I_{Na} current than epicardial and endocardial myocytes (Yan et al. 2003). The density of rapidly activating delayed rectifier potassium current (I_{Kr}) is, in turn, even across the ventricular wall (Yan et al. 2003). Alterations in the I_{Ks}:I_{Kr} ratio are attributable to TDR, thus predisposing to abnormal ventricular repolarization (Yan et al. 2003). Apparently, the preferential prolongation of action potential in M cells underlies the increase in TDR and QT interval prolongation (Shimizu et al. 2000) that has been shown to be associated with increased mortality in LQTS patients (Moss et al. 1991), in coronary artery disease patients (Schwartz et al. 1978, Puddu et al. 1986) and in the general population (Algra et al. 1991, Schouten et al. 1991, Karjalainen et al. 1997). T wave alternans refers to T wave fluctuations in morphology, amplitude, and polarity, and is associated with changes in TDR, and thus, with ventricular arrhythmias (Narayan 2006).

2 SPECTRUM OF INHERITED CARDIAC ION CHANNELOPATHIES

Following the initial description of potassium channel gene defects and their association with LQTS (Curran et al. 1995), several cardiac disorders have been demonstrated to result from ion channel dysfunction. These include purely arrhythmogenic disorders, but also diseases with apparent structural abnormalities of the myocardium (Table 1). The majority of the disorders are caused by mutated ion channels, but also defects in channels subunits and associated proteins may lead to similar disease phenotypes. A peculiar aspect of cardiac ion channelopathies is considerable overlap in genetic and phenotypic characteristics of the disorders. The phenomenon is specifically recognized in cardiac sodium channelopathies, where mutations in the sodium channel alpha subunit gene (*SCN5A*) have been shown to result in de- and repolarization abnormalities (LQTS3) (Wang et al. 1995), idiopathic ventricular fibrillation with distinct ECG patterns (Brugada syndrome 1) (Brugada et al. 1992), atrial fibrillation and dilated cardiomyopathy (McNair et al. 2004), and in a variety of conduction abnormalities (Schott et al. 1999, Tan et al. 2001, Wang et al. 2002). Interestingly, even a particular mutation in the *SCN5A* gene may lead to multiple clinical phenotypes (Kyndt et al. 2001, Grant et al. 2002, Smits et al. 2005). A few *in vitro* electrophysiological studies (Veldkamp et al. 2000, Remme et al. 2006) suggest that a specific *SCN5A* mutation may indeed result in a variety of defects, explaining part of the phenotypic diversity.

Table 1. Spectrum of cardiac ion channel disorders.

Disorder	Sub-type	Locus	Gene	Protein subunit	Functional abnormality	Reference
ATRIAL FIBRILLATION						
	1	11p15.5	KCNQ1	$K_v7.1$ α	I_{Ks} ↑	(Chen et al. 2003)
	2	21q22.1	KCNE2	MiRP1 β	I_{Ks} ↑	(Yang et al. 2004)
	3	17q23	KCNJ2	Kir2.1 α	I_{K1} ↑	(Xia et al. 2005)
	4	1q21.1	GJA5	Connexin 40*	Cell adhesion ↓	(Gollob et al. 2006)
	5	21q22.1	KCNE1§	minK β	I_{Ks} ↓	(Lai et al. 2002)
	6	12p13	KCNA5	$K_v1.5$ α	I_{Kur} ↓	(Olson et al. 2006)
BRUGADA SYNDROME						
	1	3p21	SCN5A	$Na_v1.5$ α	I_{Na} ↓	(Chen et al. 1998)
	2	3p24	GPD1L	G3PD1L*	I_{Na} ↓	(London et al. 2007)
CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA						
	1	1q42-43	RyR2	RyR2	SR Ca^{2+} leak ↑	(Laitinen et al. 2001) (Priori et al. 2001)
	2	1p13.3	CASQ2	Calsequestrin*	SR Ca^{2+} leak ↑	(Lahat et al. 2001b)
CONDUCTION DEFECTS						
		3p21	SCN5A	$Na_v1.5$ α	I_{Na} ↓	(Schott et al. 1999)
CONGENITAL SICK SINUS SYNDROME						
		3p21	SCN5A	$Na_v1.5$ α	I_{Na} ↓	(Benson et al. 2003)
FAMILIAL WOLFF-PARKINSON-WHITE SYNDROME						
		7q36.1	PRKAG2	AMPK γ_2*	I_{Na} ↑ ?	(Gollob et al. 2001)
IDIOPATHIC VENTRICULAR FIBRILLATION						
		3p21	SCN5A	$Na_v1.5$ α	I_{Na} ↓	(Akai et al. 2000)
LONG QT SYNDROME (Table 3)						
SHORT QT SYNDROME						
	1	7q35	KCNH2/ HERG	$K_v11.1$ α	I_{Kr} ↑	(Brugada et al. 2004)
	2	11p15.5	KCNQ1	$K_v7.1$ α	I_{Ks} ↑	(Bellocq et al. 2004)
	3	17q23	KCNJ2	Kir2.1 α	I_{K1} ↑	(Priori et al. 2005)

* Refer to ion channel-associated proteins. § association data of gene polymorphism with the clinical phenotype, no direct evidence of disease-causing mutations. ↓ refers to loss-of-function defect, ↑ refers to gain-of-function defect. The phenotypes of cardiac ion channelopathies are overlapping. Modified from (Lehnart et al. 2007).

3 LONG QT SYNDROME

3.1 Clinical manifestations of LQTS

Congenital long QT syndrome (cLQTS) is an inherited cardiac repolarization disorder characterized by QT interval prolongation on surface ECG and risk for ventricular tachyarrhythmias and sudden death in the presence of physical or emotional stress (Priori 2004, Schwartz 2006) or while asleep (Schwartz et al. 2001). Congenital LQTS is a rare disorder, with recent prevalence estimates of 0.01% (Ackerman et al. 2003) to 0.05% (Hofman et al. 2007). A distinct feature of LQTS is the *torsades de pointes* arrhythmia (Viskin 1999) that manifests with a twisting QRS complex around the isoelectric baseline and may develop to ventricular fibrillation and sudden death. The mean age of onset in LQTS is 12 years, but symptoms may occur from the first year of life to the late fifties and sixties (Zipes et al. 2006).

The diagnosis of LQTS is based on the measurement of the QT interval on surface ECG. The upper limit for heart rate–corrected QT interval (QTc Bazett) is 440 ms for men (Vincent et al. 1992) and 460 ms for women (Schwartz 2006). However, a significant overlap occurs in the QTc intervals of LQTS patients and healthy individuals. In a study by Vincent et al. (1992), the QTc intervals of genetically affected LQTS patients ranged from 410 ms to 590 ms, as opposed to 380 ms to 470 ms among mutation noncarriers (Vincent et al. 1992). The Schwartz scoring system (Schwartz 2006) (Table 2) is suitable for situations where the QTc interval is borderline prolonged and/or symptoms such as stress-induced syncope are absent. The Schwartz diagnostic criteria are based on repolarization abnormalities on ECG, occurrence of symptoms, and the presence of family history, providing a probability score of LQTS in a given individual. A score of ≥ 3.5 points is associated with a high probability of LQTS, and ≤ 1 point with a low probability of the disorder (Schwartz 2006).

The Schwartz criteria has high specificity (99%) but low sensitivity (19%) (Swan et al. 1998, Hofman et al. 2007), and therefore, analysis of QTc >430 ms alone with a specificity of 86% and a sensitivity of 72% has been proposed to be sufficient to distinguish mutation carriers from noncarriers (Hofman et al. 2007). In addition, exercise stress test provides information on QT interval response to physical stress, and may be used to improve the clinical diagnosis of LQTS1-2 (Swan et al. 1999b). Echocardiography is useful only in ruling out other cardiac disorders. Even though LQTS is a clinical diagnosis, constantly developing genetic testing measures enable precise molecular genetic diagnosis, and thus, provide tools for risk stratification.

Table 2. The Schwartz scoring system for LQTS 1985-2006 (Schwartz et al. 1985, Schwartz et al. 1993, Schwartz 2006)

	FINDING		SCORE
ECG	A QTc	> 480 ms	3
		460 – 470 ms	2
		450 – 459 ms (male)	1
	B Torsades de pointes*		2
	C T wave alternans		1
	D Notched T wave in 3 leads		1
	E Low heart rate for age§		0.5
CLINICAL HISTORY	A Syncope*	with stress	2
		without stress	1
	B Congenital deafness		0.5
FAMILY HISTORY^	A Family members with definite LQTS		1
	B Sudden unexplained death < 30 years among immediate family members		0.5

* Mutually exclusive, § resting heart rate below the 2nd percentile for age, ^ the same family member can not be counted in A and B.

3.2 Molecular genetics of LQTS

Clinical LQTS was first characterized in the 1950s when Jervell and Lange-Nielsen described a family with syncopal spells and prolonged QT interval in association with hearing loss (Jervell et al. 1957). However, it was not until 1995 that the first underlying cause of LQTS was identified as the mutated cardiac potassium channel gene *KCNH2* (*HERG*) (Curran et al. 1995). Today, ten genes and over six hundred mutations have been identified to cause congenital LQTS (Priori et al. 2008) (Table 3). The majority of these genes code for cardiac ion channel proteins, but also genes affecting regulatory proteins in the cardiac macromolecular assemblies underlie cLQTS (Mohler et al. 2003, Vatta et al. 2006, Medeiros-Domingo et al. 2007). LQTS is generally inherited in an autosomal dominant fashion (i.e. Romano-Ward syndrome, RWS) (Romano 1965), with the exception of extremely rare homozygote carriers of potassium channel gene mutations, which also result in congenital deafness (i.e. Jervell and Lange-Nielsen syndrome, JLNS) (Jervell et al. 1957).

Table 3. LQTS subtypes, associated genes, and functional consequences of gene defects.

LQTS subtype	Locus	Gene	Protein subunit	Functional abnormality	Reference
LQTS1	11p15.5	KCNQ1	$K_v7.1$ α	I_{Ks} ↓	(Wang et al. 1996)
LQTS2	7q35	KCNH2/HERG	$K_v11.1$ α	I_{Kr} ↓	(Curran et al. 1995)
LQTS3	3p21	SCN5A	$Na_v1.5$ α	I_{Na} ↑	(Wang et al. 1995)
LQTS4	4q25	ANK2	Ankyrin-B*	$I_{Na,K}$ ↓ I_{NCX} ↓	(Mohler et al. 2003)
LQTS5	21q22.1	KCNE1	minK β	I_{Ks} ↓	(Splawski et al. 1997)
LQTS6	21q22.1	KCNE2	MiRP1 β	I_{Kr} ↓	(Abbott et al. 1999)
LQTS7 (Andersen syndrome)	17q23	KCNJ2	Kir2.1 α	I_{K1} ↓	(Plaster et al. 2001)
LQTS8 (Timothy's syndrome)	12p13.3	CACNA1C	$Ca_v1.2$ α_{1c}	$I_{Ca,L}$ ↑	(Splawski et al. 2004)
LQTS9	3p25	CAV3	Caveolin-3*	I_{Na} ↑	(Vatta et al. 2006)
LQTS10	11q23	SCN4B	$Na_v1.5$ β4*	I_{Na} ↑	(Medeiros-Domingo et al. 2007)
JLNS1	11p15.5	KCNQ1	$K_v7.1$ α	I_{Ks} ↓	(Tyson et al. 1997)
JLNS2	21q22.1	KCNE1	minK β	I_{Ks} ↓	(Tyson et al. 1997)

LQTS1-10 refer to autosomal dominant Romano-Ward syndrome, JLNS1-2 refer to autosomal recessive Jervell-Lange-Nielsen syndrome. * refer to ion channel-associated proteins, ↓ refers to loss-of-function defect, ↑ refers to gain-of-function defect. Modified from (Lehnart et al. 2007).

3.3 Genotype-phenotype relationships

LQTS1

LQTS1 is caused by mutations in the KQT-like voltage-gated potassium channel-1 gene (*KCNQ1*) that encodes the α-subunit of the slow component of the delayed rectifier I_K current (I_{Ks}) (Roden et al. 2002). *KCNQ1* mutations have multiple consequences at the cellular level, all of them ultimately leading to a loss-of-function defect (Wang et al. 1996). *KCNQ1* proteins form functional channels as homotetramers that co-assemble with minK, the β-subunit of the I_{Ks} current, in which defects are responsible for the LQTS5 subtype (Splawski et al. 1997). LQTS1 is the most prevalent form of LQTS, accounting for up to 40-50% of the genotyped patients (Splawski et al. 2000, Tester et al. 2005b). Typically, *KCNQ1* mutation carriers present broad-based T waves on ECG (Moss et al. 1995), and the arrhythmias are triggered by physical exercise, especially swimming (Schwartz et al. 2001).

LQTS2

LQTS2 results from loss-of-function mutations of the human ether-a-go-go-related (*KCNH2*/*HERG*) gene, which codes for the α-subunit of the rapid delayed rectifier I_K channel (I_{Kr}) (Curran et al. 1995). Most QT prolonging agents block the I_{Kr} current in the cardiomyocyte, thus linking acquired LQTS with LQTS2

(Sanguinetti et al. 1995). This had led to the usage of HERG as a major target of drug safety, as pharmaceutical companies test the developmental agents for direct blockage of the HERG channel. Similarly to the I_{Ks} current in LQTS1 and LQTS5, the I_{Kr} current requires an assembly of *KCNH2* homotetramers and MiRP proteins (Roden et al. 2002). LQT2 is estimated to constitute 35-40% of clinical LQTS samples (Splawski et al. 2000, Tester et al. 2005b). Acoustic stimuli upon slow heart rate induce arrhythmias among LQTS2 patients (Schwartz et al. 2001), and a characteristic ECG reveals a low-amplitude T wave with notching (Moss et al. 1995).

LQTS3

LQTS3, the third most common cause of LQTS, arises from mutations in the *SCN5A* gene that encodes the α-subunit of the cardiac sodium channel protein (Wang et al. 1995). These gene defects lead to an abnormally persistent inward Na^+-current (I_{Na}) (Wang et al. 1995), forming the initial upstroke of the cardiac action potential. Recently, a mutation in the *SCN4B* gene coding for the β-subunit of the I_{Na} has been identified as a putative LQTS susceptibility gene (Medeiros-Domingo et al. 2007). LQTS3 patients have arrhythmias mainly while at rest, and the characteristic ECG phenotype includes peaked T waves (Moss et al. 1995).

3.4 Risk stratification

Studies based on international samples postulate that 13% of untreated LQTS patients undergo cardiac arrest or sudden cardiac death (Priori et al. 2003). The interplay between genetic defect, QTc duration, and gender has been proposed to provide an algorithm for risk stratification (Priori et al. 2003). The length of the QTc interval is the most valuable predictor of risk for cardiac events (Moss et al. 1991), and a QTc exceeding 500 ms is associated with an increased risk of symptoms by the age of 40 years, particularly in LQTS1 and LQTS2 (Priori et al. 2003). Since QTc interval duration is a dynamic measure, incorporation of follow-up ECG recordings into LQTS risk assessment has been suggested (Goldenberg et al. 2006).

LQTS patients resuscitated from cardiac arrest have a high relative risk of 12.9 of experiencing another event (Moss et al. 2000), but the risk is not directly applicable to affected family members (Zipes et al. 2006). The time-dependent history of syncope has proven to be a valuable predictor of life-threatening cardiac events among LQTS patients (Goldenberg et al. 2008a). Two or more syncopal episodes in the last two years has resulted in a 18-fold risk increase among LQTS adolescents (10-20 years) (Hobbs et al. 2006), while recent syncope resulted in a 10-fold increased risk among adults (18-40 years) (Sauer et al. 2007). After the age of 40, time-dependent syncope in addition to female gender and LQTS3 genotype remain independent risk factors for cardiac arrest/death (Goldenberg et al. 2008b).

Studies based on an International Registry for LQTS reveal that patients with LQTS1 are less likely to experience cardiac events than LQTS2 and LQTS3 patients (Priori et al. 2003). However, an earlier study by Zareba et al. reported a higher risk for cardiac events in LQTS1 and LQTS2 than in LQTS3, although lethality of these events was higher among LQTS3 patients (Zareba et al. 1998). Gender influences the probability of cardiac events. Before the age of 15, male gender is associated with a 72-85% increased risk for cardiac events in LQTS, but the gender risk is reversed in mid-adolescence (Locati et al. 1998). In general, female LQTS2 patients have a worse prognosis, whereas male gender is associated with an increased risk of cardiac events among LQTS3 patients (Priori et al. 2003). Mutational loci may play a role in the risk stratification of LQTS patients since mutations in the pore region of the *KCNH2* gene are associated with an increased risk of cardiac events as compared with nonpore region gene mutations (Moss et al. 2002). In addition, specific mutations, such as the *KCNQ1* A341V mutation, have been shown to result in an unusually severe clinical phenotype independently of the ethnicity of the LQTS family (Crotti et al. 2007).

A simplified LQTS risk stratification scheme suggests categorization of LQTS patients into high-, intermediate-, and low-risk subgroups (Moss et al. 2000, Goldenberg et al. 2008a). Secondary prevention included patients with post-CPR and spontaneous TdP and a very high (14%) 5-year Kaplan-Meier estimate for aborted cardiac arrest or sudden cardiac death, while high-risk subjects featuring either prolonged QTc >500 ms or prior syncope had a 3% risk for cardiac arrest or sudden death. Low-risk LQTS patients with QTc <500 ms and no syncopal episodes feature a 0.5% 5-year Kaplan-Meier estimate for severe cardiac events. The authors emphasize cautious interpretation of such simplified models in the dynamic clinical settings of LQTS.

3.5 Clinical management of LQTS

Due to the lack of randomized trials, recommendations for LQTS management are based on expert opinions. Such preventive measures as avoidance of QT-prolonging drugs, electrolyte disturbances, and adrenergic stimuli are essential and apply to all LQTS patients (Zipes et al. 2006). Beta-antagonists are the standard therapy for LQTS and recommended for those at increased risk of arrhythmias. In general, LQTS1 patients show a favorable response to beta-blocker therapy, while protection is only partial for LQTS2 and LQTS3 patients (Priori et al. 2004). Implantable cardioverter defibrillator (ICD) is recommended as secondary prevention for those who have been resuscitated from cardiac arrest and for those who remain symptomatic despite proper beta-blocker treatment (Zipes et al. 2006). Arrhythmias in LQTS3 are often managed with ICD in the absence of proper antiarrhythmic medication (Zipes et al. 2006). Gene-specific LQTS therapies, such as potassium-channel activators, sodium channel blockers, and protein-kinase inhibitors, may be of value in the future (Khan et al. 2004).

3.6 Finnish LQTS

Owing to few initial inhabitants, national isolation, and population bottlenecks (Peltonen et al. 1999), some LQTS mutations have enriched in the Finnish population. Apart from Finland, LQTS founder mutations have been reported in Norway (Tranebjaerg et al. 1999) and later in South Africa (Brink et al. 2005). Fodstad et al. demonstrated that four potassium channel mutations, *KCNQ1* G589D (Piippo et al. 2001), *KCNQ1* IVS7-2A>G, *KCNH2* L552S, and *KCNH2* R176W (Laitinen et al. 2000), account for 70% of the known genetic spectrum of LQTS in Finland (Fodstad et al. 2004). Thus far, *KCNQ1* G589D has been identified in 650 Finnish individuals from over 90 families presenting clinical LQTS, while the other three LQTS founder mutations have each been detected in 100-120 patients and 20 families (Swan et al., unpublished data).

In clinical samples, approximately 23-38% of the founder mutation carriers are symptomatic, and feature a mean QTc of 459-470 ms (Fodstad et al. 2004). *KCNH2* R176W, although reportedly present in 0.9% of the apparently healthy blood donors, shows significant 8% enrichment among LQTS patients (Fodstad et al. 2004). In addition, *KCNH2* R176W was identified in six *KCNQ1* G589D founder mutation carriers in six LQTS families (Fodstad et al. 2006). These compound founder mutation carriers had a mean QTc of 473 ± 30 ms, as opposed to the QTc of 457 ± 33 ms in *KCNQ1* G589D carriers (n=371) alone (Fodstad et al. 2006). The compound carriers of the two founder mutations also featured a high (67%, 4/6) frequency of symptoms (Fodstad et al. 2006). However, the role of *KCNH2* R176W as a disease-causing mutation has been questioned since it has been reported elsewhere as an innocent polymorphism (Ackerman et al. 2003, Mank-Seymour et al. 2006).

In vitro, *KCNH2* R176W results in reduced current density and slight acceleration of the deactivation kinetics (Fodstad et al. 2006). *KCNQ1* G589D channels, when coexpressed with the minK *in vitro*, show smaller currents and a rightward shift in the voltage of activation (Piippo et al. 2001). The intronic *KCNQ1* founder mutation shows a complete loss-of-function defect *in vitro*. Based on complete analysis of the coding regions of the five most common genes responsible for LQTS1-3,5,6, LQT1 is the most prevalent subtype (71%) in Finland, followed by LQT2 (23%) and LQT3 (6%) (Fodstad et al. 2004).

3.7 Genetic modifiers of LQTS

Considerable phenotypic variability occurs among the carriers of identical disease-causing LQTS mutations. Reduced penetrance in LQTS was first reported by Vincent et al. in a large LQTS kindred showing linkage to the *KCNQ1* gene in chromosome 11 (Vincent et al. 1992). Later, South African family members carrying *KCNQ1* A341V were reported to have great variability in the QTc interval and in the occurrence of symptoms (Brink et al. 2005). A number of mutation carriers showed QTc intervals within the normal range (Brink et al. 2005), providing evidence of incomplete penetrance. Apparently, both

genetic and environmental factors contribute to the reduced penetrance and variable expression of the disease, and thus, to an individual's risk for arrhythmias.

An estimated eleven million mutated single nucleotides, each with a >1% frequency, exist in the human genome (Kruglyak et al. 2001). A majority of the polymorphisms are likely to be innocent variants, with no pathophysiological consequences. However, several LQTS polymorphisms have been shown to affect the expression, localization, and function of the mutant ion channels in *in vitro* experiments (Scicluna et al. 2008). In addition, a number of LQTS polymorphisms are associated with a disease phenotype *in vivo* (Scicluna et al. 2008) or, alternatively, with QT interval duration in the general population (Table 4).

Table 4. Evidence of QT-modulating gene polymorphisms at the population level.

Gene	SNP	Study population	N	Effect of minor allele on QT interval	P	Reference
KCNH2 /HERG	K897T	Oulu	187	↑	0.005*	(Pietila et al. 2002)
		MONICA	1030	↓	0.003	(Bezzina et al. 2003)
		DESIR	398	↓	0.0055	(Gouas et al. 2005)
		KORA	3966	↓	0.0006	(Pfeufer et al. 2005)
		Framingham	2515	↓	0.01	(Newton-Cheh et al. 2007)
		FINCAVAS	1975	↑	0.011*	(Koskela et al. 2008)
		Young Finns	1894	↔	NS	(Raitakari et al. 2009)
KCNH2	rs3807375	Framingham	2123	↑	0.00006	(Newton-Cheh et al. 2007)
SCN5A	H558R	Twins	282	↓	0.03	(Aydin et al. 2005)
		DESIR	398	↑	0.0063	(Gouas et al. 2005)
KCNE1	G38S	Twins	282	↔	NS	(Aydin et al. 2005)
		KORA S4	3966	↔	NS	(Akyol et al. 2007)
	D85N	Twins	282	↔	NS	(Aydin et al. 2005)
		DESIR	398	↑	0.02	(Gouas et al. 2005)
KCNE2	T8A	Twins	282	↑	0.009	(Aydin et al. 2005)
NOS1AP	rs10494366	KORA S4	3966	↑	$1x10^{-7}$	(Arking et al. 2006)
		Framingham	1805	↑	0.004	(Arking et al. 2006)
		KORA S3	2646	↑	$1x10^{-11}$	(Arking et al. 2006)
		Rotterdam	5374	↑	$8x10^{-20}$	(Aarnoudse et al. 2007)
		Amish	763	↑	0.006	(Post et al. 2007)
		Diabetes Heart	624	↑	$6x10^{-6}$	(Lehtinen et al. 2008)
		Young Finns	1842	↑	<0.0001	(Raitakari et al. 2009)
		GRAPHIC	1837	↑	$8x10^{-7}$/0.07§	(Tobin et al. 2008)
		FINCAVAS	1924	↑	0.006/0.02§	(Tobin et al. 2008)
	rs16847548	DHS	3072	↑	0.005-$4x10^{-5}$	(Arking et al. 2009)
	rs16856785	DHS	3072	↑	0.01	(Arking et al. 2009)

* difference detected only in women. § in women/men, respectively.

KCNH2/HERG polymorphisms

The very first report of genetic modifiers in LQTS involved the *KCNH2* K897T polymorphism, which was shown to shorten the QT interval among female LQTS1 patients (Laitinen et al. 2000). The variant is relatively common, with a minor T allele frequency of 16% (Laitinen et al. 2000). Later, the same variant was proposed to promote the expression of a disease phenotype in a family with *KCNH2* A1116V mutation (Crotti et al. 2005). Discrepant results have been observed among the numerous *in vitro* electrophysiological experiments concerning the functional consequences of *KCNH2* K897T polymorphisms. K897T coexpression exaggerated the I_{Kr} reduction of the *KCNH2* A1116V mutation, corresponding to symptomatic compound carriers of the T897 allele and the mutation in the studied family (Crotti et al. 2005). In addition, the T897 allele has been shown to exhibit changes in channel expression (Paavonen et al. 2003), activation (Bezzina et al. 2003, Anson et al. 2004), deactivation (Bezzina et al. 2003, Paavonen et al. 2003), and inactivation kinetics (Paavonen et al. 2003, Anson et al. 2004), along with alterations in current density (Bezzina et al. 2003, Paavonen et al. 2003, Anson et al. 2004). During the last few years, several population-based surveys have identified the T897 allele to be associated with mild to moderate shortening of the QT interval, with a varying degree of statistical significance (Table 4) (Bezzina et al. 2003, Gouas et al. 2005, Pfeufer et al. 2005, Newton-Cheh et al. 2007). An opposite QT-prolonging effect of *KCNH2* K897T was reported in a small study of middle-aged Finnish women (Pietila et al. 2002) and later in a larger sample size from the FINCAVAS study (Koskela et al. 2008). No QT altering effect of the variant was observed in a study comprising young healthy Finns aged 24-39 years (Raitakari et al. 2009).

In addition to *KCNH2* K897T, a *KCNH2* R1047L (Larsen et al. 2001) variant has been reported to be associated with acquired LQTS (Sun et al. 2004, Mank-Seymour et al. 2006) and to alter the function of the I_{Kr} channel *in vitro* (Sun et al. 2004). Recently, genome-wide association studies have recognized intronic *KCNH2* variation rs3807375 in association with QT interval prolongation (Newton-Cheh et al. 2007).

SCN5A variations

SCN5A H558R (Iwasa et al. 2000) was first reported among Japanese LQTS patients. Afterwards, a study demonstrated the *in vitro* mitigating effect of the R558 allele on defective Na^+ channel function in a conduction disease phenotype caused by the *SCN5A* T512I mutation (Viswanathan et al. 2003). Moreover, the variant has been shown to restore intracellular trafficking of the *SCN5A* mutations underlying LQTS3 (Ye et al. 2003) and Brugada syndrome (Poelzing et al. 2006). The effect of the H558R variant on QTc interval duration was investigated by Gouas et al, who reported enrichment of the R558 allele among individuals with longer QTc interval (Gouas et al. 2005). Aydin et al. showed shorter QTc intervals among heterozygous carriers of the H558R variant (Aydin et al. 2005). The *SCN5A* S1102Y variant,

which occurs with a 13% frequency in African Americans but not in Caucasians, is associated with an increased risk for arrhythmias (Splawski et al. 2002, Burke et al. 2005) and sudden infant death syndrome (Plant et al. 2006).

KCNE1 D85N

Substantial evidence suggests that *KCNE1* D85N (Paulussen et al. 2004) may play a role as a modulator of cardiac repolarization, as it seems to cluster among both acquired LQTS patients (Wei et al. 1999b, Paulussen et al. 2004) and clinical LQTS patients with unidentified disease-causing mutation (Salisbury et al. 2006). Westenskow et al. describe a putative QT-prolonging effect of the N85 allele in 13 clinical LQTS1-2 patients (Westenskow et al. 2004). Further evidence was provided by Gouas et al. who reported increased occurrence of the variant among 200 study subjects with the longest QTc interval as compared with the 200 individuals with shortest QTc intervals in a French community-based study (Gouas et al. 2005). In addition, D85N has been shown to reduce the I_{Ks} current *in vitro* (Westenskow et al. 2004).

NOS1AP variants

Very recently, a genome-wide association study identified a novel *NOS1AP* gene as a modulator of cardiac repolarization (Arking et al. 2006). Several common *NOS1AP* variants with a minor allele frequency of 30-40% have been associated with mild to moderate QT prolongation in a total of nine independent population-based surveys with convincing statistical support (Arking et al. 2006, Aarnoudse et al. 2007, Post et al. 2007, Tobin et al. 2008, Arking et al. 2009, Raitakari et al. 2009). The *NOS1AP* gene encodes a regulator of neuronal nitric oxide synthase, and hypothetical molecular mechanism involves regulation of intra-cardiac signaling rather than a direct action on ion channel function (Arking et al. 2006).

3.8 Acquired LQTS

In addition to congenital LQTS, LQTS may also manifest as a result of extrinsic factors such as QT-prolonging medication, i.e. antiarrhythmics, antihistamines, and antipsychotics, and ischemic or structural heart disease (Roden 2004), and is therefore classified as acquired LQTS (aLQTS). Female gender, baseline QT prolongation, subclinical LQTS, electrolyte disturbances such as hypokalemia and hypomagnesemia, concomitant structural or ischemic heart diseases, and congestive heart failure, are known risk factors for aLQTS (Roden 2004). Drug-induced *torsades de pointes* (TdP) is clinically a highly relevant form of aLQTS. An estimated 1-8% of patients receiving QT-prolonging medication show QT prolongation or develop TdP (Viskin 1999). The onset of an arrhythmia is attributable to multiple hit theory (Roden 1998); the reduced repolarization reserve (Roden 2006) is further challenged by extrinsic factors resulting in electric disturbances of the heart. Owing to the similarities between congenital and

acquired LQTS, genetic susceptibility to aLQTS is anticipated. In fact, a proportion of acquired LQTS patients may present subclinical *forme fruste* congenital LQTS that will develop the disease phenotype only in the presence of extrinsic stimuli. However, a surprisingly low yield (3-6%) of disease-causing LQTS mutations have been identified in studies on aLQTS patients (Chevalier et al. 2001, Yang et al. 2002, Paulussen et al. 2004).

4 BRUGADA SYNDROME

4.1 Clinical characteristics of Brugada syndrome

Brugada syndrome was first described in 1992 as an arrhythmic disorder manifesting as elevated ST segments on the right precordial leads, right bundle branch block, and risk for ventricular fibrillation and sudden death (Brugada et al. 1992). The estimated prevalence is relatively high in Asian populations (>1/1 000) (Miyasaka et al. 2001), but significantly lower in Caucasians (Hermida et al. 2000). Noticeable ambiguity may underlie the prevalence calculations, as it is currently unknown whether a Brugada type ECG alone stands for the disease phenotype. Three forms of Brugada ECGs exist, the type 1 ECG patterns entitling a definitive diagnosis of Brugada syndrome if arrhythmias or positive family history are present (Wilde et al. 2002). If the typical ECG characteristics are not apparent, sodium channel blockers are used to augment the dysfunction of the mutated channel and unmask the concealed Brugada ECG. Analogously to LQTS3, ventricular arrhythmias in Brugada syndrome occur while at rest or asleep (Benito et al. 2008). Furthermore, male sex appears to be associated with increased risk for arrhythmias in Brugada syndrome (Gehi et al. 2006). ICD is thus far the only proven effective treatment in the disorder (Benito et al. 2008).

4.2 Molecular genetics of Brugada syndrome

Loss-of-function mutations in the α-subunit of the cardiac sodium channel encoded by the *SCN5A* gene constitute the best-characterized cause of Brugada syndrome (Chen et al. 1998). To date, over a hundred *SCN5A* mutations have been described to underlie the disorder (Antzelevitch 2007). However, only 18-30% of Brugada syndrome patients carry identifiable mutations in the *SCN5A* gene (Antzelevitch et al. 2005), suggesting noncoding mutations or genetic heterogeneity in the disease pathogenesis. In fact, a mutation in the *GPD1-L* gene encoding for glycerol-3-phosphate-dehydrogenase 1-like protein has been reported to be cosegregated with the Brugada phenotype in a large kindred (London et al. 2007). The *GPD1-L* mutation results in weaker cardiac sodium current owing to reduced cell surface expression of the sodium channels *in vitro* (London et al. 2007).

5 CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA

5.1 Clinical characteristics of CPVT

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a severe inherited arrhythmia disorder that presents with stress-induced polymorphic ventricular tachycardia in structurally normal heart. A full clinical description of the entity was provided by Leenhardt et al., who described 21 children with physical or emotional stress-induced ventricular tachyarrhythmia (Leenhardt et al. 1995). The arrhythmias appeared beyond a threshold heart rate, first as isolated monomorphic ventricular premature complexes (VPCs), and later, as heart rate increased, they developed through bigeminia to bursts of bidirectional or polymorphic ventricular tachycardia. The arrhythmias disappeared in reverse order as the triggering catecholamines or isoproterenol infusion were eliminated. In these patients, the typical symptoms included faintness, dizziness, visual disorders, and episodes of hypotonia, as well as convulsive movements and loss of consciousness in more severe attacks. Due to resemblance to ictal episodes, many of these children were initially misdiagnosed as having epilepsy. The mean age at onset was 7.8 years, and the symptoms never presented before the age of 3 years. A family history of syncope or sudden death was evident in 30% of patients.

Although no abnormalities are detectable in baseline ECG in typical CPVT, a few groups have reported bradycardia among CPVT patients (Leenhardt et al. 1995, Postma et al. 2005) similarly to the earlier findings of Leenhardt et al. (Leenhardt et al. 1995). In addition, variations in the length of the QTc interval (Swan et al. 1999a, Choi et al. 2004) have been observed. As arrhythmias only occur in the presence of catecholamines in classical CPVT, an exercise stress test is the standard diagnostic tool in unmasking the arrhythmias. However, normal treadmill test results have also been reported in symptomatic CPVT patients (Bauce et al. 2002, Priori et al. 2002a, Tester et al. 2005a). Indeed, a study by Krahn et al. suggests that epinephrine infusion may be more sensitive than exercise testing in detecting VPCs (Krahn et al. 2005). Traditionally, CPVT is defined as an arrhythmic disorder of the structurally normal heart. However, a number of reports have described minor structural abnormalities among CPVT patients (Tiso et al. 2001, d'Amati et al. 2005). The phenotypic heterogeneity of *RyR2*-linked disorders was further illustrated by a recent report by Bhuiyan et al, who describe two families with atrial arrhythmias, conduction defects and left ventricular dilatation and dysfunction due to a large genomic *RyR2* in-frame deletion (Bhuiyan et al. 2007b).

Onset of the disorder is typically in early adolescence, varying from two years (Priori et al. 2002c) to the early forties (Swan et al. 1999a), and some evidence indicates that early onset may lead to a more severe form of CPVT (Leenhardt et al. 1995). In addition, male gender, history of syncope, cardiac arrest, rapid or sustained runs of VT are associated with an increased risk for major cardiac arrhythmias (Zipes et al.

2006). A cumulative mortality rate of up to 30% has been reported by the age of 35 if CPVT is left untreated (Swan et al. 1999a).

5.2 Molecular genetics of CPVT

CPVT1

The locus of familial polymorphic ventricular tachycardia was mapped to chromosome 1q42-43 in two large Finnish families (Swan et al. 1999a), followed by studies leading to the identification of mutations in the ryanodine receptor type 2 (*RyR2*) gene. The RyR2 functions as a calcium release channel on the sarcoplasmic reticulum in cardiac myocytes. The first two reports described *RyR2* mutations in families affected by purely arrhythmogenic disorder (Laitinen et al. 2001, Priori et al. 2001), whereas Tiso et al. also detailed minor structural abnormalities of the right and left ventricle in affected individuals (Tiso et al. 2001), thus initially categorizing the disorder as ARVC/D type 2. Thus far, molecular genetic studies have revealed over seventy *RyR2* missense mutations clustering into three known 'hotspot' regions in the *RyR2* sequence: the N-terminus, the central region, and the C-terminal pore region. This is similar to the mutations in *RyR1* in malignant hyperthermia and central core disease (Marks et al. 2002). The functional significance of the clustering of *RyR2* mutations remains unclear, and no direct evidence exists of an association between mutational loci and the phenotype (George et al. 2007). The clinical phenotypes of reported *RyR2* mutation carriers include patients with CPVT, and since the disorder is associated with high mortality, also material from molecular autopsy (Tester et al. 2004). Approximately 40% of the genotyped CPVT patients carry mutations in the *RyR2* gene (Kontula et al. 2005).

CPVT2

In addition to the *RyR2*, CPVT has been linked to mutations in the *CASQ2* gene, which encodes the Ca^{2+} binding storage protein calsequestrin in the sarcoplasmic reticulum. *CASQ2* mutations cause an autosomal recessive CPVT and was first described in a Bedoin kindred from northern Israel (Lahat et al. 2001a, Lahat et al. 2001b). Later, also heterozygous carriers of a *CASQ2* mutation have been reported in association with the disease phenotype (Postma et al. 2002, de la Fuente et al. 2008). The clinical phenotype is identical to the *RyR2*-linked CPVT, but the *CASQ2*-associated recessive CPVT appears to be related to earlier onset, higher average penetrance, and poorer prognosis than the dominant form of CPVT1 (Lahat et al. 2001b, Postma et al. 2002).

Other putative disease-causing genes

CPVT presumably features genetic heterogeneity similar to that recognized among other ion channelopathies. Not only genes affecting cardiac Ca^{2+} signaling, but also genes traditionally associated

with LQTS have been linked to exercise-induced ventricular arrhythmias. Several groups have reported *KCNJ2* gene mutations underlying exercise-induced VPCs in the absence of Andersen syndrome (Tester et al. 2006, Eckhardt et al. 2007, Ruan et al. 2007). In addition, mutations in *KCNE1* underlying LQTS5 and ankyrin-B underlying LQTS4 have been reported to result in exercise-induced polymorphic ventricular tachycardia (Mohler et al. 2004, Tester et al. 2006). A recent report by Bhuiyan et al. describe a new locus 7p14-p22 for early onset autosomal recessive CPVT (Bhuiyan et al. 2007a). The candidate genes located in the region include several genes expressed in the myocardium, and identification of the mutation in the future is likely to expand the known genetic spectrum of CPVT.

5.3 Molecular mechanisms underlying CPVT

The cardiac-specific ryanodine receptor type 2 (RyR2) is a 2200-kDa tetramer located on the sarcoplasmic reticulum of the cardiac myocyte (Figure 3). The cytosolic N-terminus interacts with several regulatory proteins, such as calmodulin, calstabin, and sorcin, creating the RyR2 macromolecular complex (Zalk et al. 2007). In addition, the RyR2 receptor complex is also modulated by the luminal CASQ2 and the associated triadin and junctin (Gyorke et al. 2008). RyR2 channels are triggered by cytosolic calcium, which enters the cell following an action potential–mediated opening of the L-type calcium channels (Roden et al. 2002). Activated RyR2 channels release Ca^{2+} from the sarcoplasmic reticulum, a phenomenon known as calcium-induced calcium release (CICR) (Bers 2002).

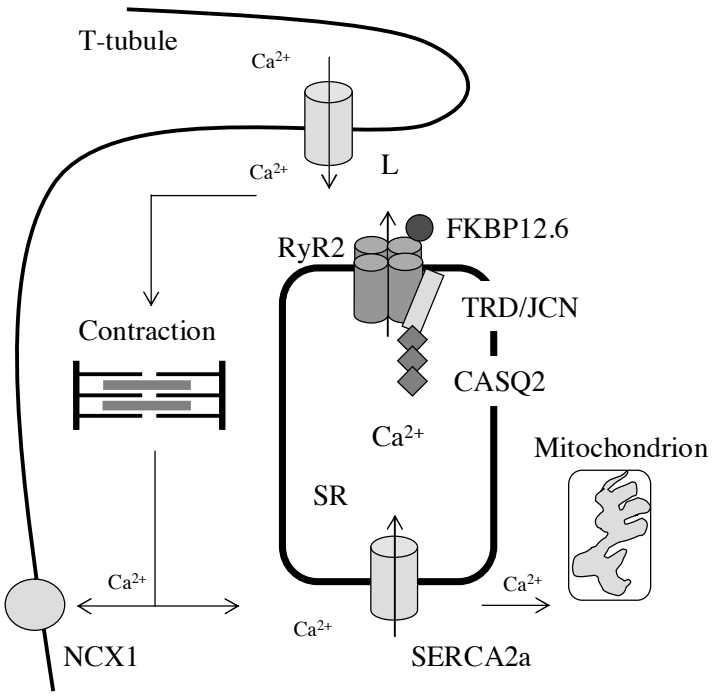

Figure 3. Cardiac calcium signaling and regulation of RyR2 channel function. L=L-type Ca^{2+} channel, SR=sarcoplasmic reticulum, FKBP12.6= calstabin, TRD=triadin, JCN=junctin, SERCA2a=sarcoplasmic reticulum Ca^{2+} ATPase, NCX=Na^{+}/Ca^{2+} exchanger.

The work by Lehnart et al. described the functional consequences of three *RyR2* mutations (P2328S, Q4201R, V4653F) originally identified in Finnish CPVT patients (Lehnart et al. 2004). Depletion of calstabin from the RyR2 receptor complex under PKA hyperphosphorylation was postulated to cause leaky RyR2 channels, and thus, delayed afterdepolarization and arrhythmias (Lehnart et al. 2004). Analogously, calstabin-deficient mice featured polymorphic VT in an exercise stress test (Wehrens et al. 2003). The arrhythmias were prevented in calstabin2$^{+/-}$ mice, but not in calstabin2$^{-/-}$ mice, by treatment with JTV19 (K201, a 1,4-benzothiazepine) (Wehrens et al. 2004), a Ca^{2+} channel stabilizer demonstrated to increase binding of calstabin to RyR2 (Lehnart et al. 2006). Furthermore, overexpression of FKBP12.6 in transgenic mice showed a marked increase in the binding of calstabin to RyR2 and prevented triggered ventricular tachycardia by reducing diastolic sarcoplasmic Ca^{2+} leakage (Gellen et al. 2008). The theory has thereafter been questioned by several groups that propose a calstabin-independent mechanism. Jiang et al. first described several CPVT-linked *RyR2* mutations that featured enhanced luminal sensitivity and resulted in store overload-induced Ca^{2+} release (SOICR) (Jiang et al. 2002, George et al. 2003, Jiang et al. 2004, Jiang et al. 2005). Interestingly, an opposite direction of action has also been reported in a CPVT-linked *RyR2* mutation, resulting in a loss of luminal Ca^{2+} activation and SOICR (Jiang et al. 2007). In addition, the role of PKA hyperphosphorylation in the CPVT pathogenesis has been called into question (George et al. 2003, Xiao et al. 2004).

The generation of CPVT mouse models has provided valuable insights into disease mechanisms. CPVT-linked transgenic mice, the *RyR2* R4496C (Cerrone et al. 2005, Liu et al. 2006, Cerrone et al. 2007) and *RyR2* R176Q (Kannankeril et al. 2006) knock-in mouse, develop bidirectional ventricular arrhythmias in response to adrenergic stimuli and show mild structural abnormalities in the latter, similarly to humans carrying the corresponding *RyR2* mutations. However, the interaction of calstabin with RyR2 in mutant *RyR2* R4496C$^{+/-}$ and R176Q$^{+/-}$ myocytes and the phosphorylation status were equivalent to those seen in nonmutant mice (George et al. 2003, Kannankeril et al. 2006, Liu et al. 2006). In addition, K201 failed to prevent ventricular arrhythmias in *RyR2* R4496C$^{+/-}$ mice (Liu et al. 2006). Plausibly, the mutational loci determine the properties of mutant *RyR2* channels that ultimately lead to an enhanced sarcoplasmic Ca^{2+} leak, and thus, to the DADs and induction of arrhythmias in CPVT. A study on *RyR2* R2474S$^{+/-}$ mice found leaky RyR2 channels in the brain to cause seizures independently of cardiac arrhythmias, suggesting combined neurocardiac nature of CPVT (Lehnart et al. 2008).

To date, seven mutations in the *CASQ2* gene have been linked to the CPVT2 phenotype. The proposed mechanisms involve alterations in the CASQ2 binding and buffering properties (Kim et al. 2007) and disrupted interaction of CASQ2 with the RyR2 receptor complex (Terentyev et al. 2006). Impaired CASQ2 buffering capacity is proposed to lead to altered sarcoplasmic free Ca^{2+} content, which is sensored by the RyR2 receptor (Gyorke et al. 2008). These mechanisms are likely to act in parallel in *in vivo* arrhythmogenesis (Gyorke et al. 2008). In murine studies, the CASQ2-null mice showed normal sarcoplasmic Ca^{2+} release and contractile function, but once exposed to catecholamines, the CASQ2-null

cardiomyocytes displayed an increased diastolic Ca^{2+} leak from the sarcoplasmic reticulum, thus contributing to catecholaminergic ventricular arrhythmias (Knollmann et al. 2006). Furthermore, a report of both $CASQ^{-/-}$ and $CASQ^{307/307}$ mice revealed adaptive changes in CASQ2 deficiency and stress-augmented RyR2 leakiness (Song et al. 2007).

5.4 Clinical management of CPVT

The standard treatment of CPVT is beta-antagonists at the maximal tolerated dose (Zipes et al. 2006). According to the Task Force on SCD in 2006 (Zipes et al. 2006), silent carriers of a *RyR2* mutation should also be treated. Although some prognostic risk factors exist for malignant arrhythmias, such as male gender and early onset, ICD is generally recommended in the secondary prevention of cardiac arrest and for patients who remain symptomatic despite maximal beta-blocker treatment. All patients with overt CPVT should avoid excess adrenergic stimuli such as strenuous exercise and swimming.

However, the efficacy of beta-blockers is not complete. Priori et al. treated *RyR2*-genotyped CPVT patients with nadolol, metoprolol, or propranolol, but 7 of 19 patients (37%) remained symptomatic (Priori et al. 2002c). Similar results were achieved in nongenotyped CPVT patients by Sumitomo et al. (Sumitomo et al. 2003). Bauce et al. (Bauce et al. 2002) and Postma et al. (Postma et al. 2005) treated 17 and 50 patients, respectively, one of whom had a fatal outcome due to noncompliance with treatment. In the Finnish patient population, three of the 39 genotyped CPVT patients died due to poor adherence to drug treatment (Swan et al., unpublished data). Considering the incomplete protection of beta-blockers in preventing sustained VTs in CPVT, better agents for clinical management of the disorder are being sought. Swan et al. found calcium channel antagonism to reduce the number of exercise-induced VPCs by 76% (Swan et al. 2005). Recently, Rosso et al. showed similar results following oral administration of combined beta-blocker-verapamil therapy (Rosso et al. 2007). In addition, experimental agents K201/JTV519 have undergone rigorous investigations and may add to the medical arsenal in the future (Wehrens et al. 2004, Wehrens et al. 2005). A recent report describes a successful surgical left cardiac sympathetic denervation of three CPVT patients (Wilde et al. 2008). The researchers propose that the procedure may offer an effective alternative treatment to CPVT patients who remain symptomatic with beta-blockers (Wilde et al. 2008).

6 SUDDEN CARDIAC DEATH

Sudden cardiac death (SCD) is defined as an unexpected death occurring shortly (<1 hour) after the onset of a change in clinical status (Zipes et al. 2006). Each year SCD claims over 300 000 lives in the United States alone (Zheng et al. 2001). The majority of SCDs occur due to complications of coronary artery disease (Virmani et al. 2001), and hence, conventional cardiovascular risk factors, such as high systolic

blood pressure, smoking, diabetes, and left ventricular hypertrophy, have proven to be potent independent risk factors of SCD at the population level (Cupples et al. 1992, Jouven et al. 1999). Only 5% of the victims of SCD are estimated to have a primary electric disorder of the heart (Zipes 2005).

First-degree relatives of SCD victims have an increased risk of sudden death, suggesting that hereditary factors contribute to an individual's risk for SCD (Jouven et al. 1999, Friedlander et al. 2002, Kaikkonen et al. 2006). Pathogenic gene variants causing familial arrhythmia disorders and/or structural heart disease are powerful predictors of SCD in a family (Priori et al. 2003). Since these disease-causing gene mutations are extremely rare in the general population, they cannot predict the risk of SCD in the background population. Previously, the common *SCN5A* S1102Y variant, unidentifiable in Caucasians, was shown to be associated with an increased risk of arrhythmias and sudden death among African Americans (Splawski et al. 2002, Plant et al. 2006), thus far being the only described ion channel gene variant with direct implications for major cardiac events at the population level. Further knowledge of population-level genetic contributors of SCD could improve management of inherited arrhythmia disorders, and, ultimately, risk stratification of the general population.

AIMS OF THE STUDY

The aim of the study was to identify common and rare ion channel gene variants underlying severe ventricular arrhythmias in clinical samples and in the general Finnish population. Specific aims were as follows:

1. To investigate the role of the four Finnish LQTS founder mutations underlying drug-induced acquired LQTS (I).
2. To estimate the prevalence of the four Finnish LQTS founder mutations in the Finnish population and to examine their effect on the QT interval at the population level (II).
3. To evaluate the effect of common gene variants on QT interval duration in the Finnish background population (III).
4. To investigate *RyR2* involvement in typical and atypical CPVT and sudden cardiac death (IV, V).
5. To identify disease-causing *SCN5A* mutations underlying Brugada syndrome in the Finnish patient population (unpublished data).

MATERIALS AND METHODS

1 STUDY SUBJECTS

In Study I, the sample consisted of 16 patients with antiarrhythmic drug–associated *torsades de pointes* arrhythmia referred to the Laboratory of Molecular Medicine, University of Helsinki, for testing of ion channel gene defects. In Studies II and III, the population was built on a two-stage stratified cluster sample of 8028 individuals from the Health 2000 Survey. The participants were compiled in September 2000 – June 2001 and represented the entire Finnish population aged ≥30 years (Aromaa 2004). DNA samples were available from 6334 individuals and digitized 12-lead ECG were recorded from 6295 study participants. Jointly, DNA samples and legible ECGs were available from 6027 individuals. A total of 959 subjects were excluded from statistical analyses due to complete left or right bundle branch block, QRS ≥ 120 ms, atrial fibrillation or flutter, pacemaker, or usage of medications affecting the QT interval. In Study III, the 27 carriers of the Finnish LQTS founder mutations were also excluded from the final analyses. In secondary analyses of Study III, individuals older than 60 years, with QTc ≥ 500 ms, history of myocardial infarction, and heart failure were eliminated.

Study IV consisted of 19 victims of sudden cardiac death referred to the Laboratory of Molecular Medicine, University of Helsinki, for genetic testing. A total of 300 ethnically matched blood donors were used as controls in determining the frequency of identified gene variants in the Finnish background population. The relatives of the deceased individuals were tested (n=64) for the identified mutations, and mutation carriers (n=20) were further clinically evaluated. Study V included 33 patients referred to the Laboratory of Molecular Medicine due to frequent ventricular ectopy of unknown origin. Altogether 232 relatives underwent cardiac examination. The occurrence of *SCN5A* mutations was investigated in six index patients referred to the Laboratory of Molecular Medicine because of syncopal spells and ECG patterns resembling Brugada syndrome (unpublished data). Written consent was obtained from all patients, and the Ethics Committee of the Department approved the studies.

2 CLINICAL EVALUATION

In Study I, the clinical records of each patient were systematically analyzed to collect data of the clinical circumstances leading to the diagnosed proarrhythmia. Resting ECGs, cardiac ultrasonography, and laboratory test results were collected before and after the documented episode of TdP. Moreover, concurrent medications were carefully evaluated to detect potential drug interactions. In Studies II and III,

clinical data were based on the patient's health questionnaire, ECG measurements, and physician's clinical examination. The mutation carriers in Study IV underwent a thorough clinical examination comprising a health inquiry, resting ECG, cardiac ultrasonography, 24-hour ambulatory ECG recording, maximal exercise stress test, and gradual intravenous epinephrine infusion 0.05 μg/kg/min-0.4 μg/kg/min for up to 20 min. The same protocol, except for the epinephrine test, was applied to participants in Study V. In the unpublished study of Brugada syndrome, relatives underwent exposition to intravenous flecainide (2 mg/kg for 30 min) if Brugada alterations were not apparent in baseline ECG.

QT intervals were determined from the onset of the QRS to the intersection of the T wave tangent to the baseline. Manual QT interval measurements on lead II were performed in Studies I, II, IV, and V. In Studies II and III, QT parameters from a 12-lead digitized ECG were measured based on a previously described algorithm (Oikarinen et al. 1998) and mean QT interval was used for final analyses. QT intervals were corrected for heart rate (QTc) utilizing Bazett's formula (Bazett 1920) (Studies I, II, IV, V). In Study III, QT intervals were heart rate-corrected with the nomogram (Karjalainen et al. 1994) derived from the same study population.

3 MOLECULAR GENETIC STUDIES

3.1 DNA extraction and polymerase chain reaction (I-V)

DNAs were derived from peripheral blood lymphocytes and extracted with either a standard phenol chloroform method (Blin et al. 1976) or a salting-out method with the Puregene DNA whole-blood purification kit (Gentra, Minneapolis, MN, USA). Amplification of genomic DNA was accomplished with polymerase chain reaction (PCR) (Mullis et al. 1986) utilizing Amplitaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) and intronic primers in screening for novel mutations.

3.2 Screening for novel mutations (IV,V)

Novel mutations in the *RyR2* (NM_001035), *FKBP1B* (NM_004116), *ATP2A2* (NM_170665), *SLC8A1* (NM_021097), and *SCN5A* (NM_198056) genes were detected by direct sequencing. Sequencing of the *CASQ2*, the integral Ca^{2+} storage protein, was excluded from Study V since the mode of inheritance in the study material did not support autosomal recessive inheritance and a previous study has failed to detect causative *CASQ2* mutations underlying CPVT in Finnish patients (Laitinen et al. 2003). Amplified DNA regions were purified using shrimp alkaline phosphatase and exonuclease 1, and sequencing reactions were completed with BigDye reagents (Applied Biosystems, Foster City, CA, USA). Automated sequencer ABI 3730 (Applied Biosystems) separated the reactions based on the incorporated di-

deoxynucleotides that terminate the elongation reaction (Sanger et al. 1977). Sequences were analyzed with Sequencer software 4.5 (Gene Codes, Ann Arbor, MI, USA).

3.3 Detection of known DNA alterations (I-V)

Detection of the known genetic alterations was performed by restriction enzyme analysis utilizing natural enzyme-specific cleavage sites on genomic DNA followed by electrophoretic separation of the resulting digestion products (Studies I, II and V, unpublished data). In Study IV, a modification of this method, i.e. primer-induced restriction enzyme analysis (PIRA) (Kumar et al. 1989), was applied. Denaturing High-Performance Liquid Chromatography (dHPLC) (Wave Nucleic Acid Fragment Analysis System HSM 3500, Transgenomic, Omaha, NE, USA) was utilized to detect of the frequency of allelic variants in controls (Studies IV and V). The method is based on DNA heteroduplex formation and their separation from homoduplexes in the Transgenomic cartridge. The temperature in the column was calculated by using the Wavemaker 4.1 software (Transgenomic). PCR products showing a divergent chromatogram profile were sequenced in the forward and reverse directions to confirm the nucleotide alteration.

The previously reported 1.1-kb *RyR2* exon 3 deletion (Bhuiyan et al. 2007b) was investigated using multiplex ligation-dependent probe amplification (MLPA) analysis of *RyR2* exon 3 (Salsa MLPA Kit P168, MRC Holland, Amsterdam, the Netherlands) and with PCR using primers located in introns 2 and 3 of the *RyR2* gene (Study V). The MLPA method is designed to detect copy number changes and uses a fluorescently labeled primer pair and multiple probes of different lengths (Schouten et al. 2002). The amplicons of the ligated probes with a target sequence in the sample DNA are separated by capillary electrophoresis, and the peak pattern is attributable to the relative quantity of the amplification product. The detailed structure of the *RyR2* exon 3 deletion was defined by direct sequencing.

High-throughput methods for detection of allelic variants were used in Studies II and III. The primary method was Sequenom MALDI-TOF mass spectrometry (MassArray Compact Analyzer, Sequenom Inc., San Diego, CA, USA) (Storm et al. 2003), which utilizes primer extension reaction chemistry in genotype characterization and allows multiplexing of SNPs to a single well. Homogenous MassEXTEND Assay is based on annealing of the hME oligonucleotide primer adjacent to the SNP and resulting allele-specific 2-3 base extension products with unique molecular masses. The more advanced iPLEX™ Assay allows more efficient genotyping up to a 36-plex level and differs from the hME Assay in utilizing single-base extension. Reactions for the MALDI-TOF assays were designed by utilizing Sequenom Assay Designer 3.1 software, and results were evaluated with SpectroAnalyzer 3.4 software. The SNP rs1805123 (Study III) did not fit a multiplex assay and was therefore genotyped independently with Applied Biosystems TagMan SNP Genotyping Assay C_11631103_10 (Applied Biosystems, Foster City, CA, USA). The assay consists of two fluorescence-labeled detectors specific for the alleles at the SNP site. The allelic

discrimination assay of the 7900HT Real Time PCR System (Applied Biosystems) measures the fluorescence signals, and SDS2.2 software (Applied Biosystems) was used in the data analysis.

4 *IN VITRO* ELECTROPHYSIOLOGICAL STUDIES

RyR2 mutagenesis was accomplished with the QuikChange II XL mutagenesis kit (Stratagene, La Jolla, CA, USA) on cassettes of h*RyR2* subcloned into pBlueScript vector. HEK293 cells were co-transfected with full-length h*RyR2* in pCMV5 vector with pCMV5/FKBP12 using a calcium phosphate precipitation technique. Microsomes containing recombinant *RyR2* were phosphorylated with the protein kinase A catalytic subunit, and phosphorylation status was confirmed in an immunoblot assay.

The single-channel recordings were conducted in planar lipid bilayers under voltage clamp conditions. Microsomes containing the recombinant *RyR2*-channels were fused to the lipid bilayer consisting of 3:1 phosphatidyl ethanolamine and phosphatidyl serine (Avanti Polar Lipids, Alabaster, AL, USA) by increasing the K^+ ion gradient across the membrane. The *cis* solution consisted of 50 mM KCl, 250 mM HEPES, 125 mM TRIS, 1 mM EGTA, and 0.5 mM $CaCl_2$ and the symmetric *trans* solution of 50 mM KCl, 53 mM $Ba(OH)_2$, and 250 mM HEPES. All experiments were carried out in pH 7.35, and free Ca^{2+} concentration was determined using the program CHELATOR (http://www.stanford.edu/~cpatton/maxc.html). Sequential addition of 20 mM $CaCl_2$ to the *cis* side was followed by 3 min of continuous recording of channel activity at each concentration. At the end of each experiment, ryanodine (5 μM) was applied to confirm the channel identity. Data were collected using AxoScope1 (Axon Instruments, Foster City, CA, USA) and analyzed by using the pClamp 6 program (Axon Instruments). Statistical analysis was performed with Student's t-test using Microcal™ Origin 6.0 software (OriginLab Corporation, Northampton, MA 01060, USA). A P-value of 0.05 was considered statistically significant.

5 STATISTICAL ANALYSES

In Studies II and III, linear regression analysis was applied to assess the size effects of genetic variants to age-, sex-, and heart rate-adjusted QT interval. The association was tested in both a one-degree (1df) additive model and a two-degree freedom (2df) general test. The prevalence estimates with 95% confidence intervals (Studies II and III) were derived from the weighted study population. Logistic regression was used in Study III to determine the odds ratio for a prolonged QT interval per each quintile increase in QT-prolonging score, which was determined by the effect of each QT-prolonging genotype relative to the reference genotype. In Studies I, VI and V, the statistical analysis was accomplished by

Student's t-test for continuous variables and Chi-square (x^2) and Fischer's exact tests for dichotomous variables. The statistical analyses were performed using SPSS 13.0/15.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

1 LQTS FOUNDER MUTATIONS IN THE FINNISH POPULATION

1.1 LQTS founder mutations underlying acquired LQTS

In Study I, 16 consecutive unrelated cases of antiarrhythmic-induced *torsades de pointes* (TdP) patients referred to the Laboratory of Molecular Medicine were screened for the four Finnish LQTS founder mutations. In addition, the clinical conditions leading to acquired LQTS were evaluated, retrospectively. The culprit drugs included amiodarone in seven, sotalol in six, flecainide in two, and propafenone in one enrolled subject. In six patients, amiodarone was administered intravenously. The mean QTc (Bazett) appeared markedly prolonged 470 ± 70 ms before drug administration and was 600 ± 70 ms at the time of TdP. However, seven individuals (44%) had a QTc < 470 ms at baseline. In addition to the baseline QT prolongation, the enrolled patients featured one or more known proarrhythmic risk factors (Roden 2004) such as female gender (n=10), hypokalemia (n=13), administration of diuretics (n=8), and congestive heart failure (n=5). Three patients (19%) carried one of the four LQTS founder mutations, which is significantly more than in the Finnish background population (Study II, $p=5.1x10^{-5}$). A *KCNQ1* G589D mutation was apparent in one amiodarone-induced TdP, while *KCNQ1* G589D and *KCNH2* L552S underlied one case each initiated by sotalol. The mean baseline QTc of the mutation carriers was 480 ± 20 ms, and corresponding QTc at the time of proarrhythmia was 540 ± 40 ms. None of these three patients reported a history of syncope before the drug challenge.

1.2 LQTS founder mutations in the Finnish background population

A large population-based cohort of 6334 Finnish individuals aged 30 or more was utilized in Study II to estimate the actual prevalence of the four LQTS founder mutations in the Finnish background population. Of the 6334 individuals genotyped over the course of the study, founder mutations were identified in 27; *KCNQ1* G589D in eight, *KCNQ1* IVS7-2A>G in one, *KCNH2* L552S in two, and *KCNH2* R176W in 16 individuals. Collectively, these four LQTS founder mutations constitute a prevalence estimate of 0.4% (95% CI 0.3-0.6%) in the Finnish population. As the original Health 2000 cohort was a cluster sample, detailed maps of the origins of these founder mutations in the Health 2000 Study and in the clinical samples were constructed to ascertain that the clinical LQTS families had not become overrepresented in the Health 2000 study material (Study II, Supplementary data).

To evaluate the QT-prolonging effect of these founder mutations, a total of 984 individuals were excluded due to the extrinsic factors potentially affecting the length of QT interval or the accuracy of the QT interval measurement technique. The mean QTc interval among founder mutation noncarriers was 396 ± 22 ms, while the founder mutation carriers had a QTc of 423 ± 30 ms (n=25, two founder mutation carriers were excluded due to usage of potential QT-prolonging drugs, $p=1.26x10^{-9}$). The *KCNQ1* G589D resulted in a mean QTc interval of 435 ± 40 ms, *KCNH2* L552S 438 ± 26 ms, and *KCNH2* R176W 430 ± 20 ms in the Finnish background population (Table 5). In linear regression analysis, the mean QT intervals were adjusted for age, sex, and heart rate. The mutant allele A of *KCNQ1* G589D was associated with a 50.5-ms increase in the adjusted QT interval compared with mutation noncarriers (SE 7.1, 2.7 SD, $p=8.99x10^{-13}$). The minor *KCNH2* R176W allele resulted in a 22.2-ms increase in the adjusted QT interval compared with noncarriers (SE 4.7, 1.2 SD, $p=2.15x10^{-6}$). Systematic ascertainment of arrhythmias was not part of the Health 2000 Study. One mutation carrier of *KCNQ1* G589D with a QTc of 520 ms had previously been symptomatic.

Table 5. Clinical characteristics of the founder mutation carriers (n=25) and noncarriers (n=4758) after exclusions.

	KCNQ1 G589D n=7	*KCNH2* L552S n=2	*KCNH2* R176W n=16	Noncarriers n=4758
Females /males	4/3	1/1	8/8	2524/2234
Age, years	43 ± 7 (34-57)	38 ± 2 (36-39)	53 ± 11 (30-70)	51 ± 14 (30-97)
HR, bpm	55 ± 5 (48-63)	60 ± 16 (49-71)	61 ± 7 (50-72)	63 ± 11 (34-120)
QT, ms	451 ± 33 (406-507)	442 ± 32 (420-465)	414 ± 33 (357-480)	388 ± 30 (279-536)*
QTc, ms	435 ± 40 (403-520)	438 ± 26 (420-457)	430 ± 20 (396-459)	396 ± 22 (323-514)†

Data are presented as mean ± SD (range), unless otherwise indicated. HR=heart rate, bpm= beats per minute, QTc = QT interval corrected for heart rate using Bazett's formula. * refers to $p=2.17x10^{-10}$ and † to $p=1.26x10^{-9}$ in comparison of QT parameters among founder mutation carriers and noncarriers. The excluded carrier of *KCNQ1* G589D had a QTc of 438 ms and the carrier of *KCNQ1* IVS7-2A>G a QTc of 409 ms. Because of failures in genotyping the individual mutations, the genotype numbers do not add up to a total of 5068 individuals.

2 COMMON GENE VARIANTS MODIFY QT INTERVAL AT POPULATION LEVEL

In Study III, the population-based Health 2000 material was utilized to test the association of common LQTS and *NOS1AP* gene variants with QT interval duration in the Finnish background population. Nonsynonymous LQTS variants were selected if they were identifiable in the Finnish population based on previous studies of clinical LQTS samples (Fodstad et al. 2004) and showed evidence of a functional role in disease pathogenesis. In addition, recently characterized *NOS1AP* variants and an intronic *KCNH2* variant with apparent QT-prolonging effects in other population samples were included in the study. The results of linear regression analysis showing the effect of each SNP on age-, sex-, and heart rate (Nc) -

adjusted QT interval are presented in Table 6. The *KCNE1* D85N minor allele with a frequency of 1.4% is associated with a 10.5-ms prolongation of the adjusted QT interval (SE 1.6, 0.57 SD, $p=3.6x10^{-11}$). Accordingly, the mean QT_{Nc} among major homozygotes was 393 ± 20 ms (n=4684), while the D85N heterozygotes had a mean QT_{Nc} of 404 ± 20 ms (n=127) and minor allele homozygotes a mean QT_{Nc} of 415 ± 23 ms (n=3). In agreement with previous studies, the modest QT-prolonging effect of *KCNH2* rs3807375 (effect size 1.6 ms, SE 0.4, $p=5.4x10^{-5}$) and several correlated *NOS1AP* variants were replicated in the Health 2000 material. The strongest *NOS1AP* association was observed with rs2880058, with a 4.0-ms (SE 0.4) prolongation of the adjusted QT_{Nc} interval per each minor G allele ($p=3.2x10^{-24}$). The *KCNH2* K897T variant showed a modest 2.6-ms shortening in adjusted QT_{Nc} interval per each minor C allele ($p=2.1x10^{-7}$).

Table 6. Effect of SNPs on age-, sex-, and heart rate (Nc) -adjusted QT interval in the Health 2000 Study.

Gene	SNP	Genotypic model				Allelic model		
		Heterozygote	Minor homozygote	*P* 2 df	R^2	Per minor allele	*P* 1 df	R^2
KCNH2	rs3807375	2.1 (0.12)	2.9 (0.16)	$1.4x10^{-4}$	0.004	1.6 (0.08)	$4.7x10^{-5}$	0.004
KCNH2	K897T	-2.6 (-0.14)	-4.9 (-0.27)	$1.4x10^{-6}$	0.006	-2.6 (-0.14)	$2.1x10^{-7}$	0.006
KCNH2	R1047L	-0.5 (-0.03)	-10.8 (-0.58)	$4.0x10^{-3}$	0.002	-1.5 (-0.08)	$4.9x10^{-2}$	0.001
SCN5A	R190G	-0.5(-0.03)	-	$8.5x10^{-1}$	0.000	-0.5(-0.03)	$8.5x10^{-1}$	0.000
SCN5A	H558R	1.4 (0.08)	3.1 (0.17)	$6.6x10^{-3}$	0.002	1.5 (0.08)	$2.0x10^{-3}$	0.002
SCN5A	A572D	0.3 (0.02)	7.1 (0.39)	$7.2x10^{-1}$	0.000	0.5 (0.03)	$6.6x10^{-1}$	0.000
KCNE1	D85N	10.5 (0.57)	20.6 (1.12)	$3.1x10^{-10}$	0.009	10.5 (0.57)	$3.6x10^{-11}$	0.009
KCNE1	G38S	0.6 (0.03)	0.7 (0.04)	$5.1x10^{-1}$	0.000	0.4 (0.02)	$2.9x10^{-1}$	0.000
KCNE2	T8A	0.1 (0.01)	-	$9.8x10^{-1}$	0.000	0.1 (0.01)	$9.8x10^{-1}$	0.000
NOS1AP	rs2880058	4.5 (0.24)	7.7 (0.41)	$2.3x10^{-23}$	0.021	4.0 (0.22)	$3.2x10^{-24}$	0.021
NOS1AP	rs4657139	4.5 (0.24)	7.5 (0.41)	$4.9x10^{-23}$	0.022	4.0 (0.22)	$9.0x10^{-24}$	0.021
NOS1AP	rs10918594	3.9 (0.21)	6.5 (0.35)	$2.2x10^{-22}$	0.021	3.9 (0.21)	$8.7x10^{-23}$	0.020
NOS1AP	rs10494366	4.0 (0.22)	6.6 (0.36)	$4.6x10^{-18}$	0.016	3.5 (0.19)	$8.3x10^{-19}$	0.016

Values are differences from major homozygotes in milliseconds. Beta coefficients standardized to the SD of the age-, sex-, and nomogram-adjusted residuals are shown in parentheses. The standard deviation of age-, sex-, and nomogram-adjusted QT residuals is 18.39.

To assess the clinical impact of the four QT-modulating SNPs, *KCNE1* D85N, *KCNH2* rs3807375, *KCNH2* K897T, and a *NOS1AP* rs2880058, we composed a QT-prolonging score by using the beta coefficients from the 2 df model as weights. The score calculates the predicted QT effect for each individual based on their genotype. A score of 0 results for a study subject who carriers none of the alleles associated with QT interval prolongation. A one-point increase in the QT-prolonging score was associated with a 0.89-ms increase in the adjusted QT_{Nc} interval ($p=4.6x10^{-38}$). We also divided the study material into quintiles of a continuous QT-prolonging score and found a 2.4-ms increase in the age-, sex-, and heart rate-adjusted QT interval for each quintile increase in score ($p=1.6x10^{-32}$). The mean QT_{Nc} in the first quintile was 388 ± 19 ms, while the mean QT_{Nc} in the fifth quintile was 398 ± 20 ms ($p=8.3x10^{-23}$).

3 *RYR2* MUTATIONS IN SUDDEN CARDIAC DEATH

Upon studying 19 consecutive cases of sudden cardiac death referred by Finnish forensic medicine physicians to the Laboratory of Molecular Medicine for genetic analyses, two novel *RyR2* missense mutations were identified in direct sequencing of the coding regions of the gene. Neither of the mutations were identifiable in 600 control alleles from randomly collected blood donors.

The *RyR2* G2145R, located in the vicinity of the central mutational hot spot region, changes the polarity and creates a positive charge. A victim of sudden cardiac death, aged 41 years, had experienced a syncopal event related to exercise (Figure 4, Family A). The medicolegal autopsy provided no apparent explanation for the sudden death. The 23-year-old daughter of the index patient carried the *RyR2* mutation, but did not feature abnormalities upon clinical examination. The *in vitro* analysis of the *RyR2* G2145R in single-channel recordings showed statistically significantly enhanced open probability to cytosolic Ca^{2+} under basal conditions at 1μM [Ca^{2+}] compared with wild-type RyR2 channels (Figure 5). The PKA phosphorylated RyR2 G2145R channels exhibited similar open probabilities to the phosphorylated wild-type RyR2 channels.

The mutation *RyR2* R3570W was identified independently in two victims of sudden death (Figure 4, Families B, C). This mutation is located in exon 75 of the *RyR2* gene, where disease-causing mutations have not previously been identified. The index patient in Family B died suddenly at the age of 17 years while playing volleyball, and the index patient in Family C at the age of 55. In both victims, medicolegal autopsy revealed a moderately enlarged and dilated heart of over 500 g and a slightly hypertrophic (12-15 mm) left ventricular wall. Histology did not show myofibrillar disarray, and the main coronary arteries were normal in both men.

In the *RyR2* R3570W families, molecular genetic analyses were conducted on 64 family members, 20 of whom appeared to carry the familial *RyR2* defect. All R3570W heterozygotes also carried intronic variant

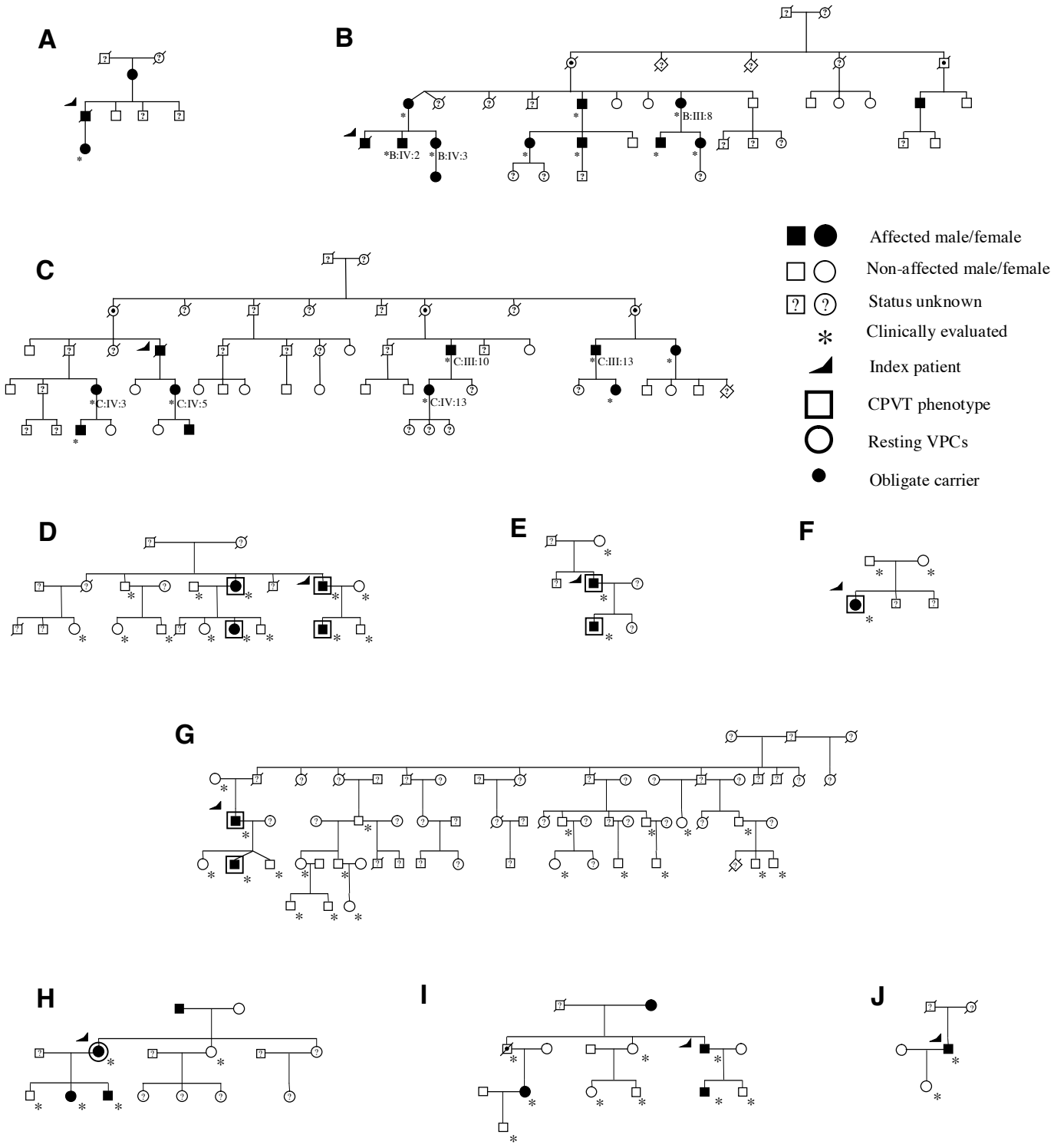

Figure 4. Pedigree data on families with *RyR2* missense mutations (A-C, F-H), *RyR2* exon 3 deletions (D,E), and *SCN5A* mutations (I,J) (Studies IV and V, unpublished data). Family A=*RyR2* G2145R, B and C=*RyR2* R3570W, D and E= *RyR2* exon 3 deletion, F=*RyR2* S616L, G=*RyR2* R1051P, H=*RyR2* N3308S, I=*SCN5A* E1784K, J=*SCN5A* IVS21+16G>A.

IVS74-3C>G unidentifiable in controls that resides in close proximity to the amino acid-altering mutation in exon 75. A total of 17 *RyR2* R3570W mutation carriers were available for further clinical examination. None of the relatives reported syncopal events. Altogether, four carriers showed similar mild structural changes of the left ventricle as the two deceased individuals (Table 7). Interestingly, ventricular premature complexes (VPCs) occurring in resting conditions were evident in two living carriers. The 34-year-old sister of the index patient in Family B featured recurrent VPCs at rest, which were largely suppressed during exercise. Holter recording revealed over 5000 polymorphic VPCs and a salvo of three beats. *In vitro* single-channel recordings showed a significant gain-of-function defect for the mutant native *RyR2* R3570W channels in the range of lower diastolic to upper systolic cytosolic Ca^{2+} concentrations (Figure 5).

Table 7. Range of abnormalities in surviving *RyR2* R3570W mutation carriers.

Individual	Gender	Age (years)	ECG	ECHO	Exercise test	Epinephrine test	24 h Holter
B:III:8	Female	65	VPCs	LVEDD 62 mm	8 VPCs	Negative*	640 VPCs
B:IV:3	Female	34	VPCs	Normal	46 VPCs	N/A	5080 VPCs
B:IV:2	Male	40	Normal	LVEDD 62 mm Septum 12 mm	Normal	N/A	N/A
C:III:10	Male	64	Normal	Septum 12 mm	Normal	Negative	190 VPCs
C:III:13	Male	64	Normal	Septum 14 mm	Normal	Negative	2 VPCs
C:IV:3	Female	48	Normal	Normal	6 VPCs	Negative	5 VPCs
C:IV:13	Female	43	Normal	Normal	Normal	Positive	170 VPCs

LVEDD = left ventricular end-diastolic volume, VPC = ventricular premature complex. N/A= not available. * refers to the normal epinephrine test, please see Methods.

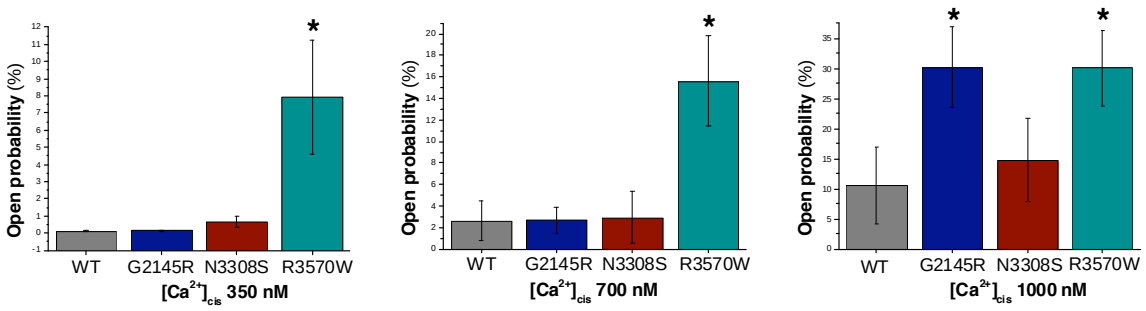

Figure 5. Open probabilities of wild-type RyR2 (n=8), mutant RyR2 G2145R (n=9), N3308S (n=8), and R3570W (n=12). *p<0.05

4 CARDIAC CALCIUM CYCLING GENE MUTATIONS IN FAMILIAL VENTRICULAR ARRHYTHMIAS RESEMBLING CPVT

A total of 33 symptomatic patients with frequent VPCs of unknown etiology were recruited to Study V. The criteria for enrollment were as follows: frequent VPCs occurring during exercise and consecutive VPCs, history of syncope, and/or sudden juvenile death in the family. None of the patients had structural heart disease or prolonged QT interval. In 16 patients, VPCs were inducible solely upon exercise, while in 17 patients, VPCs occurred also at rest and at the recurring phase of exercise, thus differing from classical CPVT. In both subgroups, an index patient had suffered from juvenile (≤ 40 years) sudden cardiac death. Over the course of the study, 232 relatives underwent thorough clinical examination. Familial occurrence of the disease phenotype was evident in 38% (n=6) and 41% (n=7) of the two subgroups.

The analysis of the exon 3 region of the *RyR2* gene revealed two 1.1-kb genomic deletions in two CPVT patients. The 33-year-old index patient in Family D (Figure 4) showed frequent bidirectional VPCs during exercise typical of CPVT. Three family members had a similar symptomatic CPVT phenotype. Interestingly, atrial fibrillation was observed in the index patient's son and sister, but not in other relatives. The sister also featured atrioventricular conduction abnormalities during follow-up. DNA analysis revealed a 1.1-kb deletion of the *RyR2* gene (c.168-301_c.273+722del1128), predicted to result in the elimination of the highly conserved exon 3 and thus 35 amino acids (p.Asn57-Gly91) from the *RyR2* protein. The 39-year-old index patient in Family E featured atrial fibrillation and multifocal VPCs during the exercise stress test and developed increased trabeculation of the left ventricle suggestive of noncompaction cardiomyopathy. His son showed a similar phenotype in the absence of atrial arrhythmias and cardiomyopathy. The two patients carried the previously reported 1.1-kb deletion (c.168-228_c.273+793del1126) (Bhuiyan et al. 2007b), which is expected to result in an in-frame deletion of 35 amino acids from the RyR2 protein similarly to the deletion identified in Family D.

The direct sequencing of the *RyR2* gene revealed two novel *RyR2* mutations, S616L and R1051P, underlying typical CPVT. The *RyR2* S616L mutation arose de novo in a female adolescent (Family F, Figure 4), while the *RyR2* R1051P was identified in a 35-year-old father and his son (Family G, Figure 4). The 35-year-old index patient also featured lateral ST alterations in ECG, but only a minor septal hypertrophy of 13 mm in the echocardiography. In addition, a novel *RyR2* variant N3308S, detectable in one of the 600 control alleles, was evident in a 34-year-old female patient with frequent resting VPCs (Family H, Figure 4). She presented with over 20 000 predominantly single VPCs, but also salvos of up to four successive beats in the 24-h ECG recording. Two of the descendants carried the *RyR2* N3308S, but showed no abnormalities in clinical evaluation. The single-channel recordings of the *RyR2* N3308S did not reveal enhanced probability to cytosolic Ca^{2+} compared with the wild-type channels (Figure 5).

As the majority of patients recruited to the study did not have disease-causing mutations in the *RyR2* gene, the probands were screened for putative candidate genes that affect calcium cycling in the cardiac myocyte, and could therefore result in a disease phenotype identical to CPVT. The direct sequencing of genes coding for calstabin/FKBP12.6 (*FKP1B*) and sodium-calcium exchanger/NCX1 (*SLC8A1*) did not reveal any genetic variants leading to amino acid alterations. A novel polymorphism, T982M, was identified in the *ATP2A2* gene encoding the sarcoplasmic reticulum Ca^{2+} ATPase, also known as SERCA2a, with a nonsignificant frequency difference among probands and controls.

5 SURVEY OF *SCN5A* MUTATIONS IN BRUGADA SYNDROME

Of the six patients featuring ECG patterns suggestive of Brugada syndrome, two were identified to carry mutations in the *SCN5A* gene (unpublished data). The mutation *SCN5A* E1784K was identified in a 51-year-old index patient (Family I, Figure 4), who was resuscitated from ventricular fibrillation. The baseline ECG showed a typical type 2 Brugada ECG with a 2-mm ST segment elevation in precordial leads V1-V2 followed by positive T waves. The QTc interval was prolonged to 530 ms. Altogether, three relatives carried the same mutation and featured varying degrees of cardiac abnormalities in the clinical evaluation. The index patient's son, aged 22 years, had a similar type 2 Brugada ECG pattern with biphasic T waves in precordial leads and QTc interval prolongation of 460 ms. The other son featured normal baseline ECG and ST segment elevation upon exposure to flecainide, but the electrophysiological studies were normal. The index patient's brother, whose DNA was not available, had died suddenly and unexpectedly at the age of 30. The daughter of the deceased brother featured no evidence of Brugada syndrome in clinical examinations, but showed an abnormal QTc interval duration of 490 ms in baseline ECG and excess prolongation of the QT interval in the 24-h ECG recording at slower heart rates, suggestive of a LQTS3 phenotype. The mother of the index, also a carrier of the familial gene defect, was unavailable for clinical examination. Patients featuring clinical Brugada syndrome or LQTS3 have been treated with ICDs. In Family J (Figure 4), the only affected individual showed Brugada-type ECG with ST segment elevation, normal QTc interval, paroxysmal atrial fibrillation, and sinus node dysfunction, and carried splice site mutation *SCN5A* IVS21+16G>A, predicted to result in disruption of the correct splicing in the corresponding mRNA.

DISCUSSION

1 PREVALENCE OF LQTS IN FINLAND

LQTS is generally a rare disorder, with recent prevalence estimates of 0.01% (Ackerman et al. 2003) to 0.05% (Hofman et al. 2007). In Finland, the four previously identified founder mutations, *KCNQ1* G589D, *KCNQ1* IVS7-2A>G, *KCNH2* L552S, and *KCNH2* R176W, have been shown to explain up to 40-70% of the genetic spectrum of congenital LQTS (Piippo et al. 2001, Fodstad et al. 2004). Therefore, it is hardly surprising that these mutations also underlie a considerable proportion of cases with antiarrhythmic-induced *torsades de pointes* arrhythmias, as shown in Study I. The presented 19% frequency of disease-causing LQTS gene mutations underlying acquired LQTS is considerably more than the previously reported frequency of 5% in other populations (Yang et al. 2002, Paulussen et al. 2004) and is likely to reflect the genetic composition of Finnish people. However, the limited study sample precludes any statements regarding the prevalence and nature of concealed congenital LQTS in drug-induced TdP in Finland. Nevertheless, the Study I does provide further evidence that congenital LQTS gene mutations may underlie acquired LQTS. In agreement with earlier studies, up to 40% of the study subjects showed a QTc < 470 ms before drug exposure, suggesting that the risk for proarrhythmia is not always predictable from baseline ECG.

Taking advantage of the unique constellation of inherited LQTS in Finland, we aimed at estimating the actual prevalence of LQTS founder mutations using a large population-based sample of over 6000 individuals representing the entire population of Finland aged 30 years or more. The calculated prevalence estimate of 0.4% is striking and suggests that 20 000 of the 5.2 million inhabitants in Finland may be genetically predisposed to severe ventricular arrhythmias. Even after excluding the 16 carriers of *KCNH2* R176W with a milder QT-prolonging effect, the prevalence estimate of 0.2% (95% CI 0.1-0.3%) is considerably more than generally expected. As the study population did not include subjects younger than 30 years and the analysis was targeted only to the four founder mutations, the actual prevalence of LQTS is likely to be even higher. This finding is not only of national interest, but provides an example for population-based screening to identify individuals with a potential risk of arrhythmias. Since the analyzed LQTS founder mutations are specific to the Finnish population, the results cannot be extrapolated to other populations.

In addition to the prevalence estimates, the effect sizes of mutant alleles on QT interval prolongation were assessed. The described effect sizes of 22-50 ms are substantial, but caution must be employed in translating this information to arrhythmia susceptibility. Data on LQTS risk stratification have derived from clinical LQTS samples only, and therefore, no direct evidence of the causality of these mutant

alleles to ventricular arrhythmias exists at the population level. In the clinical LQTS founder mutation samples, the mean QTc intervals are somewhat higher (Fodstad et al. 2004) than in the carriers from the Health 2000 Study. Logically, the overt congenital LQTS correlates with longer QT intervals, but clinical LQTS patients may also possess other as yet unidentified genetic factors that affect the QT interval, and thus, the disease phenotype. Despite their potential to cause a life-threatening form of LQTS, the Finnish founder mutations may lead to milder phenotypic effects than disease-causing LQTS mutations in general. Of the founder mutation carriers in clinical samples and their genetically screened relatives, 23-38% are symptomatic (Fodstad et al. 2004), but the proportion is likely to decline as more distant, probably asymptomatic relatives are evaluated. In addition, the penetrance of the founder mutations is incomplete, ranging from 21% to 34% (Fodstad et al. 2004) (Laitinen et al. 2000) in the clinical LQTS families.

The *KCNH2* R176W mutation requires particular attention since it has been reported as an innocent polymorphism in other populations (Ackerman et al. 2003, Mank-Seymour et al. 2006). The updated information on the 112 Finnish *KCNH2* R176W mutation carriers identified to date reveal symptoms in 16 (14%) of the mutation carriers and a prolonged QT interval in 40% of male and 22% of female mutant allele carriers (Study II). Previously, the *KCNH2* R176W has also been identified in compound heterozygous carriers of the *KCNQ1* G589D mutation, with longer QTc intervals and more frequent arrhythmia symptoms than the *KCNQ1* G589D carriers alone (Fodstad et al. 2006). The 22-ms effect size of the *KCNH2* R176W on the age-, sex-, and heart rate-adjusted QT interval and the estimated frequency of 0.3% in the Finnish population are in accordance with previous studies that have suggested that *KCNH2* R176W is a potentially disease-causing population-prevalent modifier of Finnish LQTS (Fodstad et al. 2004). Considering the substantial QT-prolonging effects of these LQTS founder mutations in the background population, it is evident that these individuals are at increased risk of developing arrhythmias if the repolarization reserve (Roden 2006) is further challenged by exposure to QT-prolonging medication and hypokalemia, in particular (Roden 2004).

2 COMMON GENETIC MODIFIERS OF CARDIAC REPOLARIZATION

KCNE1 D85N

Previously, *KCNE1* D85N has been shown to be associated with acquired LQTS in patients with unrecognized disease-causing mutations in other LQTS genes (Wei et al. 1999b, Paulussen et al. 2004, Mank-Seymour et al. 2006). In addition, the minor N85 allele has been shown to be associated with prolonged QTc interval in a limited sample of LQTS1 and LQTS2 patients (Westenskow et al. 2004). Further evidence is provided by Gouas et al, who studied 200 individuals featuring the longest and shortest QTc intervals in an original study population of nearly 4000 French individuals (Gouas et al.

2005). They reported increased odds of the minor N85 allele carriers being in the longer QTc interval, but the significance level remained borderline (p=0.03) considering the number of SNPs tested in the study. Study IV provides convincing evidence that the *KCNE1* D85N variant, present in 2.6% of the Finnish population, is associated with QT interval duration and results in a 10-ms increase in the age-, sex-, and heart rate-adjusted QT interval per each minor allele copy.

KCNH2/*HERG* variants

In addition to the *KCNE1* D85N association, Study III replicated the QT-modulating effect of two *KCNH2* variants. After the initial description of the *KCNH2* K897T (Laitinen et al. 2000), marked attention has been raised to fully understand the consequences of the polymorphism. Several population-based studies have reported associations of the SNP with QT interval shortening, with varying degrees of statistical significance (Bezzina et al. 2003, Gouas et al. 2005, Pfeufer et al. 2005, Newton-Cheh et al. 2007). Conflicting results also exist (Pietila et al. 2002, Koskela et al. 2008). A limited study sample of randomly selected middle-aged healthy Finnish women shows an opposite QT-prolonging effect of the minor T897 allele (Pietila et al. 2002). Furthermore, compound carriers of the *KCNH2* T897 allele and a *KCNQ1* G589D mutation showed longer QT intervals than mutation carriers with the wild-type *KCNH2* K897 allele during a maximal exercise stress test (Paavonen et al. 2003). The intronic *KCNH2* rs3807375 was previously shown to be associated with increased QT duration among participants of the Framingham Heart Study (Newton-Cheh et al. 2007). In addition, this SNP shows linkage to the QT-prolonging variant *KCNH2* rs3815459 (Pfeufer et al. 2005). Taken together, the majority of the available data suggest that *KCNH2* K897T has a QT interval-shortening effect in baseline conditions, while the intronic *KCNH2* rs3807375 variant appears to modulate the baseline QT interval in the opposite direction in the general population.

NOS1AP variants

Common genetic variants in the *NOS1AP* gene coding for the neuronal nitric oxide synthase regulator CAPON were first demonstrated to be associated with QT interval duration in the German-based genome-wide KORA study (Arking et al. 2006). Several *NOS1AP* variants have subsequently been reported to be associated with modest QT interval prolongation in ten independent population-based samples (Arking et al. 2006, Aarnoudse et al. 2007, Post et al. 2007, Tobin et al. 2008, Arking et al. 2009, Raitakari et al. 2009)(Study IV) and also in multiethnic pedigrees showing enrichment of type 2 diabetes (Lehtinen et al. 2008). The evidence based on genetic association studies is convincing, but the molecular mechanisms of brain-enriched CAPONs on myocardial repolarization remain largely unknown. Very recently, Chang et al. showed that CAPON is expressed in the myocardium, interacts with NOS1, and accelerates cardiac repolarization by inhibition of L-type calcium channels and resulting enhancement of I_{Kr} (Chang et al. 2008). NOS1 has also been demonstrated to affect cardiac contractility (Barouch et al. 2002, Burkard et al.

2007, Oceandy et al. 2007), and CAPONs may also have other as yet unidentified biological effects on cardiac ion channels (Chang et al. 2008).

Other LQTS variants of uncertain significance

In Study III, effect sizes of several LQTS variants on QT interval duration did not reach statistical significance. These variants, including *KCNH2* R1047L, *SCN5A* R190G, *SCN5A* A572D, *KCNE1* G38S, and *KCNE2* T8A, seemingly do not modulate baseline QT interval duration in the Finnish population. In addition, the null effects of *KCNE1* G38S (Akyol et al. 2007, Gouas et al. 2007) and *KCNE2* T8A (Pfeufer et al. 2005) have been confirmed in other populations. However, whether these variants act on cardiac repolarization upon exposure to other extrinsic factors, such as exercise, hypokalemia, ischemia, or QT-prolonging medications, remains obscure (Roden 2004).

In addition, the effect of *SCN5A* H558R on QT interval under basal conditions remains unsettled. Since *SCN5A* contributes to a set of ion channelopathies, the *SCN5A* H558R has been studied in clinical patient populations featuring lone atrial fibrillation (Chen et al. 2007) and the Brugada syndrome (Poelzing et al. 2006). Its effect on cardiac repolarization at the population level is somewhat unclear, as the only existing evidence comes from the French DESIR study with borderline statistical support (p=0.01) (Gouas et al. 2005). Considering the nature of the genetic association studies with multiple SNPs, the need for p-values less than 10^{-5} or 10^{-6} is widely acknowledged (Newton-Cheh et al. 2005). Therefore, Study III cautiously interprets the existing evidence of the *SCN5A* H558R association with QT interval with a p-value of 10^{-3} to be inconclusive.

Clinical impact of common genetic variants

Despite the significance levels, the actual allelic effects of the common *KCNH2* K897T and rs3807375 as well as the *NOS1AP* rs2880058 variant remain relatively modest, as reflected by the low R2 values of the linear regression models. Presumably, these common variants cannot individually account for the population burden of QT prolongation or the increased risk of arrhythmias. In addition, extrapolating these results to other populations must be done cautiously since the association studies have been performed in study samples of European ancestry only. The construction of the QT-prolonging score attempted to estimate the combined effect of these SNPs on QT interval in addition to the *KCNE1* D85N variant. The 10-ms difference between the QT intervals of the first and fifth quintiles of the QT-prolonging score is comparable with the QT-prolonging effect of drugs withdrawn from the market by pharmaceutical companies (De Ponti et al. 2002), and thus, of potential clinical relevance. Since QT interval prolongation is associated with increased mortality in patients with coronary artery disease (Schwartz et al. 1978, Puddu et al. 1986) and in the general population (Algra et al. 1991, Schouten et al.

1991, Karjalainen et al. 1997), even small additive changes caused by common variants may contribute to increased risk of arrhythmias at the population level.

3 PHENOTYPIC AND GENOTYPIC VARIABILITY IN CPVT-RELATED DISORDERS

3.1 Exon 3 deletion in the *RyR2* gene

The identification of two analogous 1.1-kb *RyR2* exon 3 deletions in two patients with CPVT phenotype (Study V) suggests that this region may be of importance in the pathogenesis of inherited arrhythmia disorders. The corresponding in-frame deletion of 35 amino acids in the *RyR2* protein was previously characterized by Bhuiyan et al. in two families with exercise-related ventricular arrhythmias, atrial arrhythmias, conduction defects, and left ventricular dysfunction (Bhuiyan et al. 2007b). Our patients featured a classical CPVT phenotype in the absence of consistent evidence for *RyR2*-related atrial arrhythmias and/or structural abnormalities. However, the identification of *RyR2* exon 3 deletions independently in altogether four kindreds thus far suggests that this region may provide a target for molecular genetic studies in CPVT-related disorders.

3.2 *RyR2* missense mutations

Upon investigating 19 cases of sudden cardiac death in Study IV, two novel *RyR2* mutations were identified in deceased individuals. The clinical evaluation of *RyR2* mutation carriers in family members revealed phenotypes not typical for CPVT. The only available carrier of *RyR2* G2145R did not feature any abnormalities in the maximal exercise stress test, which, however, does not exclude the possibility that this mutation is a cause of classic CPVT. The penetrance of CPVT-linked *RyR2* mutations is high, up to 80-90%, but not complete (Swan et al. 1999a).

In addition to *RyR2* G2145R, *RyR2* R3570W, detected independently in two deceased individuals, showed divergent phenotypic profiles among the mutation carriers. First, the two deceased individuals featured left ventricle dilatation and mild hypertrophy at autopsy, which were also apparent in a milder form in four surviving relatives. Previously, CPVT has been classified as an arrhythmogenic disorder of an intact myocardium, to distinguish it from arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D), which, in addition to ventricular tachycardia, features enlargement of the ventricles and replacement of myocardium by fibrofatty tissue (Nava et al. 1988). In fact, it has been suggested that these diseases represent allelic disorders (Tiso et al. 2001). However, the finding of the integral role of desmosomal genes in ARVC pathogenesis (Sen-Chowdhry et al. 2007) implies that *RyR2*-linked ARVD2 represents CPVT with mild structural abnormalities.

As cited above, a recent study of two kindreds featuring exercise-related ventricular arrhythmias, atrial fibrillation, conduction defects, and occasional left ventricular dysfunction revealed a 1.1-kb genomic *RyR2* deletion (Bhuiyan et al. 2007b). Interestingly, one of the study subjects featured ventricular arrhythmias in resting conditions (Bhuiyan et al. 2007b). Similarly, in the extended pedigree analysis of the *RyR2* R3570W mutation carriers, two surviving relatives featured ventricular arrhythmias unrelated to exercise. In addition, Study V revealed a rare *RyR2* N3308S variant with no demonstrable *in vitro* effect on *RyR2* channel activity in a patient with frequent VPCs in resting conditions. The clinical significance of the variant remains uncertain, but *RyR2*-linked cardiac disorders evidently represent extended phenotypes from resting and exercise-induced ventricular arrhythmias to varying degrees of structural abnormalities of the heart.

Since the clinical findings of the surviving *RyR2* R3570W mutation carriers were scanty, the causality of the gene defect in sudden cardiac death and in the low-penetrant expression profiles of the surviving mutation carriers remains disputable. Identification of amino acid-altering mutation *RyR2* R3570W independently in two victims of SCD in a gene linked to a highly malignant arrhythmia disorder makes hypothesis of a causal role justifiable. However, since the carriers of the mutation appeared to be distant relatives, other genetic factors contributing to the SCD cannot be ruled out. This further challenges the risk assessment of the surviving *RyR2* R3570W mutation carriers that do not represent overt CPVT. In the era of high-throughput genotyping, the results of Study IV emphasize the value of comprehensive molecular genetic and clinical assessments of mutation carriers and available family members.

Several other limitations apply to Studies IV and V. Neither study was designed to define the prevalence of *RyR2* gene mutations in SCD and other CPVT-related disorders. The investigated phenotypes are rare and the resulting small sample sizes preclude any definitive statements of the occurrence of rare mutations in these candidate genes in other study populations. Since Study IV reports the results of victims of SCD referred by different forensic medicine physicians, data may be adversely affected by individual definition and referral bias. Despite the uniform inclusion criteria of Study V, the survey represents a heterogeneous group of patients with frequent VPCs of unknown etiology. Nevertheless, elucidating the genetic background of this heterogeneous group is essential so that the severe forms of the disorder can be identified.

3.3 Other genes affecting cardiac calcium signaling

An average of 40% of the CPVT patients carry identifiable *RyR2* mutations (Kontula et al. 2005). It was hypothesized that mutations in *FKBP1B*, *ATP2A2,* or *SLC8A1* could explain the genetics of CPVT patients lacking mutations in the coding regions of the *RyR2* gene. Several *in vitro* and murine experiments have supported the role of depleted FKBP12.6 in generating a gain-of-function defect in

mutant ryanodine channels and therefore delayed afterdepolarizations (DADs) and arrhythmias (Lehnart et al. 2004). However, controversial findings support a calstabin-independent mechanism where a Ca^{2+} overload in the sarcoplasmic reticulum leads to Ca^{2+} spillover waves via *RyR2* channels (Jiang et al. 2002, George et al. 2003, Jiang et al. 2004, Xiao et al. 2004, Jiang et al. 2005). The abnormal Ca^{2+} cycling and sarcoplasmic reticulum content in CPVT pathogenesis imply that also SERCA2a/*ATP2A2*, playing a central role in cardiac excitation-contraction coupling, could be a plausible candidate gene for severe arrhythmic disorders. In addition, a zebrafish model provides evidence that loss-of-function mutations in cardiac *SLC8A1* encoding the sodium-calcium exchanger (NCX1) may lead to intracellular Ca^{2+} imbalance irrespective of membrane depolarization or catecholaminergic stimulus (Langenbacher et al. 2005). Due to the limited sample size in Study V, the pathogeneity of these integral cardiac calcium signaling genes in other study populations cannot be ruled out. However, a majority of the patients featuring frequent VPCs of unknown etiology did not exhibit mutations in *RyR2*, *FKBP1B*, *ATP2A2,* and *SLC8A1* genes in this study, thus reflecting the complex genetic background of CPVT-related disorders.

3.4 Heterogeneity of *in vitro* effects of mutant RyR2 channels

The single-channel recordings of the mutant RyR2 G2145R revealed an increased open probability to upper systolic cytosolic Ca^{2+} concentration over 1 μM [Ca^{2+}]. The gain-of-function defect occurs independently of PKA hyperphosphorylation and calstabin depletion. Similarly, the RyR2 R3570W resulted in a leftward shift in the Ca^{2+}-dependence curve without the effect of circulating catecholamines. The gain-of-function defect persisted from the physiologic lower diastolic to upper systolic Ca^{2+} concentrations. Presumably, the arrhythmia-triggering Ca^{2+} leak via SCD-linked RyR2 mutant channels occurs due to a relative Ca^{2+} overload of the sarcoplasmic reticulum. Alternatively, the persistent sarcoplasmic reticulum Ca^{2+} leak may activate other Ca^{2+} signaling pathways, resulting in the mild cardiomyopathy observed among carriers of *RyR2* R3570W. Since a number of SCD-linked *RyR2* mutation carriers did not feature abnormalities in the clinical evaluation, other predisposing factors are necessary for a lethal event to occur. Male gender may be a contributing factor, consistent with suggestions of previous studies on CPVT (Priori et al. 2002c). The present data reinforce earlier assumptions that the mutant *RyR2* channels result in multiple functional consequences *in vitro*, and that the mutational locus may be one determinant of these effects.

4 PHENOTYPIC VARIABILITY OF SPECIFIC *SCN5A* MUTATIONS

The *SCN5A* E1784K is the most common single LQTS3 mutation, and it has previously been identified to cause LQTS3 in a Finnish family (Fodstad et al. 2004) and in multiple families worldwide (Wei et al. 1999a, Splawski et al. 2000, Tester et al. 2005b). It has also been reported to underlie Brugada syndrome in a single Caucasian family (Priori et al. 2002b). Very recently, a multicenter study aimed to determine

whether diverse biophysiological properties explained the variety of clinical phenotypes encountered among *SCN5A* E1784K carriers (Makita et al. 2008). In evaluation of a cohort of 15 kindred and 44 *SCN5A* E1784K mutation carriers, the majority of study participants (93%) showed a LQTS3 phenotype, but overlapping Brugada syndrome (22%) and sinus node dysfunction (39%) were also present (Makita et al. 2008). The delicate *in vitro* analyses revealed that the *SCN5A* E1784K mutation leads to a negative shift of the steady state of the Na^+ channel inactivation and to enhanced tonic block of sodium channel antagonists that explain the extended phenotypes among the mutation carriers (Makita et al. 2008). These *in vitro* functional results are congruent with previous reports showing similar functional defects in other *SCN5A* mutations, resulting in the mixed phenotypes (Bezzina et al. 1999, Veldkamp et al. 2000, Viswanathan et al. 2001). The identification of the *SCN5A* E1784K mutation in a Finnish family showing characteristics of Brugada syndrome and QT interval prolongation typical of LQTS3 (Marjamaa et al., unpublished data) provides further evidence that the mixed phenotypes are common in *SCN5A* channelopathies and may occur even within a single family.

5 GENETIC TESTING OF INHERITED ARRHYTHMIA DISORDERS

The last decade of molecular genetics has provided a valuable tool for clinicians in diagnostics of inherited arrhythmia disorders. Genetic screening of family members potentially at risk of developing a disease phenotype enables identification of individuals at the early, often pre-symptomatic phase. This is of particular interest in inherited ventricular tachyarrhythmia syndromes, such as LQTS and CPVT, where clinical follow-up and lifestyle modifications, such as avoidance of certain medications, electrolyte disturbances, and strenuous exercise, can effectively avert risk for sudden cardiac death (Zipes et al. 2006). The three most common LQTS genes responsible for LQTS subtypes 1-3 are estimated to account for 75% of the genetic spectrum of LQTS (Splawski et al. 2000, Tester et al. 2005b). However, genetic screening at the population level has not been considered practical owing to the genetic complexity and rarity of inherited arrhythmia disorders, and thus, the high costs of screening.

Since LQTS is enriched in the Finnish population and the four Finnish LQTS founder mutations account for the majority of the known genetic spectrum of the disorder, population screening of LQTS founder mutations may become cost-effective in Finland along with the rapidly developing genetic screening measures. In addition, identification of the minor allele carriers of the *KCNE1* D85N, leading to a significant QT-prolonging effect in the general population, could mark individuals at increased risk for repolarization-related arrhythmias. As evidenced in the Study III, the four polymorphisms showing statistically significant QT-modulating effects (i.e. *KCNH2* K897T, *KCNH2* rs3807375, *KCNE1* D85N, and NOS1AP rs2880058) collectively account for a potentially important QT interval prolongation and could therefore be useful in identifying individuals at increased arrhythmia susceptibility. The exon 3

region of the *RyR2* gene may provide a new target for genetic testing in clinical samples resembling CPVT.

CONCLUSIONS

The four Finnish LQTS founder mutations *KCNQ1* G589D, *KCNQ1* IVS7-2A>G, *KCNH2* L552S, and *KCNH2* R176W are surprisingly prevalent in the Finnish population. These mutations lead to considerable QT interval prolongation also at the population level and may thus contribute to increased risk of repolarization-related arrhythmogenesis. This unique enrichment of LQTS in Finland provides excellent research opportunities to study the genetic modifiers and environmental factors contributing to the divergent clinical phenotypes of the mutant allele carriers. In addition, the limited number of enriched variants provides a rationale for assessing whether genetic testing and early identification of the mutant allele carriers could become cost-effective for the Finnish healthcare system.

KCNE1 D85N minor allele, present in 1.4% of the Finnish population, is associated with a considerable QT interval prolongation in the general population, and could thus identify individuals at increased risk for arrhythmias in the background population, but may also represent a modifier factor of clinical LQTS. *KCNH2* K897T, *KCNH2* rs3807375 and *NOS1AP* rs2880058 variants were confirmed to show a modest QT-modulating effect at the population level, similarly to other populations.

RyR2 missense mutations are detectable in a series of sudden cardiac death patients. However, not all *RyR2* mutations lead to a typical CPVT phenotype with exercise-induced ventricular tachycardia in a structurally normal heart. Large genomic *RyR2* deletions comprising exon 3 provide a target for future genetic studies in *RyR2*-mediated arrhythmia disorders.

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